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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Anthony	Krantis	Ottawa, Ontario, Canada

☐ *Additional inventors are being named on the ___ separately numbered sheets attached hereto.*

TITLE OF THE INVENTION (280 characters max)

Methods and Compositions For Regulating Gut Motility And Food Intake

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

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☒ Firm *or* Individual Name	Leon R. Yankwich, Esq.				
Address	YANKWICH & ASSOCIATES				
Address	130 Bishop Allen Drive				
City	Cambridge	State	MA	ZIP	02139
Country	U.S.A.	Telephone	(617) 491-4343	Fax	(617) 491-8801

ENCLOSED APPLICATION PARTS *(check all that apply)*

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Respectfully submitted,

SIGNATURE Leon R Yankwich 7/6/99 Date 07 06/ 99 /

TYPED or PRINTED NAME Leon R. Yankwich REGISTRATION NO. *(if appropriate)* 30,237

TELEPHONE (617) 491-4343 Docket Number: QGN-006.0 PRV

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METHODS AND COMPOSITIONS FOR REGULATING GUT MOTILITY AND FOOD INTAKE

General Field of the Invention

This invention is generally in the field of treating obesity and regulating food intake. In particular, this invention relates to methods of regulating food intake in which trichothecenes, analogs thereof, or purinergic compounds are administered to alter gut motility and thereby satiety. The invention also relates to methods of screening for analogs of trichothenes and also for agonists and antagonists of purinergic receptors, which are useful for regulating food intake.

Background of the Invention

Overeating leading to obesity is a major health problem. Obesity increases the risk of diabetes, heart disease, cancer and other chronic diseases, in addition to the physical or mechanical restrictions imposed on the body. Although such adverse health effects of obesity are scientifically well documented and generally well understood by the public, the effective control of appetite and overeating on an individual basis has been a goal difficult to attain for millions of people. Approximately 25 percent of the children in North America are considered overweight or obese. North Americans alone spend approximately $40 billion per annum on weight loss treatments, and this amount appears to be increasing. A recent study conservatively estimated the annual cost of treating obesity in Canada at $1.8 billion, representing 2.4 percent of the total health care expenditures for all diseases (see, "Cost of Obesity: $1.8 billion," in *Pharmaceutical Manufacturers Association of Canada*, March 1999, page 11).

Currently available anti-obesity drugs work for the most part by targeting central nervous system (CNS) pathways to induce appetite suppression. However, such drugs have a number of

CNS-related side effects, such as anxiety, and there is the potential for chronic health problems such as hypertension, cardiovascular disease, and diabetes. Another current approach to treating obesity is to control appetite by using "bulk" products, which are ingested instead of normal food. Such bulk products have the problem of altering nutritional status in that the bulk product does not contain the necessary range of desirable nutrients. Moreover, the individual who ingests a bulk product may refuse to consume any food, even desirable nutrients.

Drugs that suppress appetite are among the least desirable means to treat obesity because weight is usually regained once administration of such drugs is halted. Furthermore, serious undesirable side effects, including increased risk of diseases such as primary pulmonary hypertension, may limit the use of such drugs. For example, the appetite suppressants fenfluramine and dexfenfluramine were recently pulled off the market by their manufacturers because of a potential for serious adverse effects on the lungs and heart.

Another type of obesity treatment that has emerged recently is the use of drugs that interfere with fat absorption from the small intestine. Such a drug may, for example, inhibit pancreatic enyzmes used for fat digestion. Undigested fat is then passed through the intestines and excreted. Decreasing fat absorption can result in oily stool, oily spotting of undergarments, intestinal gas, frequent bowel movements, and decrease absorption of fat-soluble nutrients such as vitamins A, D, and E. The long-term usefulness of inhibitors of fat absorption to treat obesity and the acceptability of such drugs by patients remain unclear.

There is currently no medical approach that cuts weight gain without unhealthy side effects or increased risk of disease. Needs remain for effective treatments for obesity and methods of controlling weight gain in humans and other animals without untoward nutritional and medical side effects.

Summary of the Invention

This invention provides methods of treating obesity and controlling food intake in humans and other animals. The invention is based on the discovery of how mycotoxin trichothecenes produce food or feed refusal in humans and other vertebrate animals and also on the elucidation of the neural circuitry regulating patterns of gut motor activity (gut motility), which propels food through the gut organs. The methods of treatment described herein involve administering a compound that affects the pattern of gut motility, that is, the pattern of contractions, relaxations, and quiescence of the smooth muscle tissue of the organs of the gut. Stimulating the "fed pattern" of gut motor activity signals satiety, that is, a feeling of fullness, which shortens the time an individual spends eating or feeding. Thus, compounds that stimulate the "fed pattern" of gut motility are useful in methods of treatment where the goal is to limit food intake as in treating obesity. Compounds that stimulate the "fasting pattern" or prolong the onset of the fed pattern of gut motility will tend to increase eating or feeding time because satiety is not signaled to the body. Such compounds are particularly useful in methods of increasing weight gain in animals raised as commercial sources of meat and poultry.

Methods of treating obesity provided by the invention comprise administering a trichothecene mycotoxin, which stimulates the fed pattern of gut motility. Preferably, the methods of treating obesity comprise administering a trichothecene from the nivalenol-related group of compounds consisting of nivalenol, 4-deoxynivalenol (DON), trichothecolon, trichothecin, 3-acetydeoxynivalenol, 7-acetyldeoxynivalenol, 3,15-diacetyldeoxynivalenol, 4-acetylnivalenol(fusarenon-X), and 4,15-diacetylnivalenol. More preferably, the methods of treating obesity comprise administering the trichothecene DON at a dose which is non-toxic and

non-emetic, but which stimulates the fed pattern of gut motility in the individual. The trichothecene may be administered by any of a variety of routes, including orally or parenterally. A trichothecene may be injected, for example, intravenously, intra-arterially, or intra-muscularly. Preferably, a trichothecene is administered orally in the methods of the invention.

Alternatively, methods of treating obesity comprise administering a trichothecene analog, which is a compound that functions like a trichothecene to stimulate the fed pattern of gut motility. Trichothecene analogs may be structurally related to or structurally distinct from trichothenes. Thus, trichothecene analogs may be derived from a trichothecene such as DON or may be any of a variety of compounds, including inorganic compounds, other organic compounds, such as amino acids, peptides, polypeptides, proteins, nucleotides, nucleic acids, carbohydrates, lipids, and combinations thereof.

In another embodiment, the invention provides methods and compositions for regulating gut motility and treating obesity by administering a compound that binds P_{2X1} purine receptors (purinoceptors), which are directly involved in regulating the fed pattern of gut motility. In particular, an agonist of the P_{2X1} purine receptor is a purinergic compound that binds the receptor to stimulate the fed pattern of gut motility. Stimulating the fed pattern of the gut motor activity signals satiety and thereby shortens feeding time and food intake. Preferably, the agonist of the P_{2X1} receptor that is useful in treating obesity according to the invention is a "non-desensitizing" agonist in that molecules of the agonist are able to bind the P_{2X1} receptor and to stimulate the P_{2X1} mediated fed pattern of gut motility, without eventually blocking or inactivating the receptor. In a more preferred embodiment, the non-desensitizing agonist of the P_{2X1} receptor is a sructural analog of ATP.

In another embodiment of the invention, a method of increasing weight in a human or other animal comprises administering a desensitizing agonist or an antagonist of the P_{2X1} receptor. A desensitizing agonist or an antagonist compound useful in such methods of the invention binds and blocks the P_{2X1} receptor. Blocking the P_{2X1} receptor will inhibit the fed pattern of gut motility and/or prolong the fasting pattern of gut motility, which in turn increases feeding time and food uptake because satiety is not sensed. Such methods are particularly useful in raising commercial livestock for market.

In another aspect of the invention, the non-desensitizing agonists or the antagonists of the P_{2X1} receptor are administered by any of a variety of routes, including orally, parenterally, and, if desired, topically to the gut tissue where the receptor resides.

In still another aspect of the invention, methods are provided for identifying a trichothecene analog compound, which stimulates the fed pattern of gut motility in an animal, comprising: testing the compound for the ability to inhibit protein synthesis, and then testing that such a compound stimulates gut motility. The ability of a compound to regulate (stimulate or inhibit) patterns of gut motility can be measured using an *in vitro* gut bath assay, an *ex vivo* gut organ assay, or an *in vivo* assay. Trichothecene analogs that are identified by such screening methods and are able to stimulate the fed pattern of gut motilty may be used to treat obesity according to the methods of the invention.

In another embodiment of the invention, methods are provided for identifying agonists and antagonists of the P_{2X1} receptor by testing compounds for the ability to bind the P_{2X1} receptor *in vitro* or *in vivo*. Compounds that bind the P_{2X1} receptor may then be further tested for the ability to regulate gut motility. A compound is used in a particular method of the invention depending on whether the compound stimulates or inhibits a particular pattern of gut motility.

Brief Description of the Drawings

Figure 1 shows a schematic diagram of the *in vivo* set up of Krantis et al. (*Can. J. Physiol. Pharmacol., 74*: 894-903 (1996)), employed for recording gastrointestinal motility in anaesthetized experimental animals, such as a rat. Foil strain gauges are attached by a glue to selected sites of gut organs, for example, the serosal surface of the gastric antrum, proximal duodenum, or distal ileum, along the longitudinal muscle layer. Wire leads are attached to an IBM computer data acquisition system.

Figure 2 shows a schematic representation of the neural pathways controlling fed and fasting patterns of gut motility in the duodenum and ileum. The arrangement of cholinergic (ACh), nitrergic (NO), purinergic (ATP), and VIPergic (VIP) neurons is shown together with the different receptor targets and/or inputs: mus. (cholinergic muscarinic), 5-HT_3 (serotonergic), nic. (cholinergic nicotinic), P_{2X} (purinergic). A plus sign ("+") indicates a stimulatory input between neurons, and stimulation and contraction at smooth muscle of the gut; a minus sign ("-") indicates an inhibitory input. DON = deoxynivalenol, stimulator of gut hyperactivity (fed pattern) and satiety. NO = nitric oxide, a non-adrenergic, non-cholinergic (NANC) inhibitory transmitter in the proximal duodenum and also of the propagatory P_{2X}-purinergic and cholinergic (muscarinic, mus.) motor activity in duodenum and ileum. VIP = vasoactive intestinal peptide, activator of nitrergic prejunctional inhibition of motor innervations. ATP = adenosine triphosphate, de-sensitizing agonist of purinergic receptors, such as, P_{2X} receptors. ACh = acetyl choline, the cholinergic chemical signal that binds at muscarinic (mus.) receptors to excite motor neurons. 5-HT = 5-hydroxytryptamine (serotonin), binds to 5-HT_3 (serotonergic) receptors on neurons and is the major transmitter of enteric interneurones mediating neurogenic

stimulation of NANC relaxations and cholinergic contractions of the smooth muscle of the gut. nic. = cholinergic nicotinic receptor of neurons.

Figure 3 shows the chemical structure of 4-deoxynivalenol (DON).

Figure 4 shows a recording of the spontaneous motor activity of the rat gastric antrum in a control animal showing the oscillatory appearance of contractile and relaxant responses. Vertical marks indicate time (t) at 0 and 50 minutes after start of recording. Administration of DON (first arrow to right of t = 0 minutes) at 10 $mg \cdot kg^{-1}$, i.v., abruptly attenuated the motor activity of gastric antrum. Within 40 minutes, the control motor pattern recovered, however, a proximal readministration of DON (second arrow) was without effect.

Figure 5 shows an example of a recording of the *in vivo* motility pattern of the rat duodenum control activity. The spontaneous *in vivo* motility pattern of the duodenum control activity (no DON) consists of periodic "grouped" (G) and "intergroup" (I) activity. Vertical marks indicate time (t) at 0, 30, 120, and 150 minutes after start of recording. The first arrow after t = 30 minutes, indicates systemically administered DON (arrow) at 10 mg/kg (i.v.), which induced a sustained hyperactivity (46 ± 15 min). Following recovery of motor activity to control level, readministration of DON (arrow after t = 150 minutes) was without effect.

Figures 6A-6D show data from a quantitative analysis of the effects L-NAME and α, β-methylene ATP on frequency (Freq) and amplitude (Amp) of DON induced relaxations in the rat duodenum (Figure 6A, frequency, and Figure 6B, amplitude) and ileum (Figure 6C, frequency, and Figure 6D, amplitude). Grouped motor activity in the presence of L-NAME and DON (broadly spaced diagonal bars) was equivalent to that with DON alone (closely spaced diagonal bars). α, β-methylene ATP significantly attenuated the frequency and amplitude of DON

induced relaxations (filled bars) to the level of control intergroup activity (no DON, open bars), in the duodenum (n=8) and ileum (n=4).

Figures 7A-7D show a quantitative analysis of the effects of the 5-HT_3 receptor antagonist, granisetron, on spontaneous and DON induced activity in the rat duodenum. Granisetron (i.v. or i.a., broadly spaced diagonal bars) selectively attenuated the frequency (Freq) and amplitude (Amp) of "grouped" relaxations (n=6) (Fig. 7A, frequency, and Fig. 7B, amplitude) and contractions (n=3) (Fig. 7C, frequency, and Fig. 7D, amplitude), however, it did not alter the stereotypic DON induced hyperactivity (compare closely spaced diagonal bars (DON alone) with filled bars (DON + granisetron)).

Figure 8 is a bar graph showing the effects (as percent of control) of α,β-methylene ATP on DON-enhanced motor activity for contractions and relaxations of the duodenum in piglets. "Control" represents the group of piglets that received no DON and no α,β-methylene ATP. "DON" represents the group of piglets that received DON only (1 $mg \cdot kg^{-1}$). "α,β-methylene ATP + DON" represents the group of piglets that received intra-arterial injection of α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a.) during DON (1 $mg \cdot kg^{-1}$) enhanced motor activity of the duodenum. Control group values were set as 100 %. All other values are percent of control values. Open bars represent average (4 piglets) amplitude of relaxations. Filled bars represent average (5 piglets) frequency of relaxations. Open cross-hatched bars represent average (3 piglets) amplitude of contractions. Close cross-hatched bars represent average (2 piglets) frequency of contractions. "ψ" indicates $p < 0.05$ compared to control. "ϕ" indicates $p<0.05$ compared to DON enhanced activity.

Figure 9 is a bar graph showing the effects (as percent of control) of α,β-methylene ATP on DON-enhanced motor activity (contractions and relaxations) of the ileum in piglets.

"Control" represents the group of piglets that received no DON and no α,β-methylene ATP. "DON" represents the group of piglets that received DON only (10 $mg \cdot kg^{-1}$). "α,β-methylene ATP + DON" represents the group of piglets that received intra-arterial injection of α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a.) during DON (10 $mg \cdot kg^{-1}$) enhanced motor activity of the ileum. Control group values were set as 100 %. All other values are per cent of control values. Open bars represent average (4 piglets) amplitude of relaxations. Filled bars represent average (5 piglets) frequency of relaxations. Open cross-hatched bars represent average (3 piglets) amplitude of contractions. Close cross-hatched bars represent average (2 piglets) frequency of contractions. "ψ" indicates $p < 0.05$ compared to control. "ϕ" indicates $p<0.05$ compared to DON enhanced activity.

Figure 10 shows a schematic representation of the arrangement of cholinergic, nitrergic, GABAergic, purinergic and VIPergic neural elements within the proposed tonic and modulatory pathways controlling spontaneous motor activity in the rat duodenum and ileum.

Detailed Description

Compositions and methods of treating obesity and regulating food intake by modulating the motor activity of gut organs in humans and other vertebrate animals are described. These methods are based on the discovery that trichothecene compounds, such as 4-deoxynivalenol (DON), stimulate the pattern of contractions and relaxations in organs of the gut that normally occur when food is ingested. Stimulation of this "fed pattern" of gut motility signals satiety, that is, the feeling of fullness, which is an important factor that affects the time that an individual spends eating. Trichothecenes, such as DON, act at a site outside the organs of the gut and send a signal, which is sent down neural pathways leading to the smooth muscle of the gut organs. A

specific receptor, the purinergic receptor P_{2X1}, present on cells of the smooth muscle of the small intestine has been identified which is involved in regulating specific aspects of gut motor activity. Accordingly, compounds that act as agonists or antagonists of the P_{2X1} receptor are also useful in regulating gut motility and to control satiety and time spent eating.

In order to accurately describe the invention the following terms are defined.

As used herein, "gut" refers to the gastrointestinal tract consisting of the stomach, small intestine, and large intestine.

As used herein, "gut motility" or "gut motor activity" refers to the motor behavior of the smooth muscle in the gastrointestinal organs (stomach, small intestine, and large intestine) of humans and other animals which activity consists of periods of alternating muscular contractions and relaxations, as well as periods of quiescence or relatively little activity. For example, in normal, healthy humans and other animals, the frequency and amplitude of muscular contractions and relaxations of the small intestine become heightened when food is ingested in order to propel food aborally (forward) into the intestines for nutrient extraction and absorption (see, "fed pattern" of gut motility, below). Other patterns of gut motility may occur depending on the presence or absence of food in various parts of the gut organs. Furthermore, the proximal portion of a particular gut organ may exhibit motor behavior that differs from the activity in a distal portion of the organ, such as in the case of the duodenum (the beginning portion of the small intestine) and the ileum (the terminal portion of the small intestine).

As used herein, the "fed pattern", "fed pattern activity", and "segmentation" are synonymous and refer to the continuous pattern of contractions and relaxations of the small intestine of the gut in an animal, including humans, that normally occurs as the result of ingesting food. The fed pattern of gut motility propels ingested food through the gut for nutrient

extraction and absorption, and eventually excretion of unabsorbed material as waste. The fed pattern of gut motor activity typically begins within minutes of ingesting food and is responsible for signaling satiety, that is, the feeling of fullness. Thus, satiety from the fed pattern of gut motility normally informs an individual that eating can be ended. Satiety is sensed by an individual via the fed pattern of gut motility long before the brain has an opportunity to analyze the nutrient content of the blood; a separate process, which takes place hours after food has been consumed and which is responsible for signaling cravings for specific nutrients, such as, proteins, carbohydrates, salt, and fats, which are maintained at specific levels for health.

The fed pattern is both characteristic and different for each organ and even different sites in the same organ of the gut. In the small intestine, the fed pattern is characterized by a continuous series of contractions and relaxations of the smooth muscle, which mixes intestinal contents, propels food aborally into the intestines, and delays anterograde propulsion to enhance substrate absorption (Lundgren et al., *Dig. Dis. Sci.*: 264-283 (1989)). This fed pattern activity replaces the "fasting pattern" of gut motor activity (see, below), which occurs after food has been propelled through the gut for nutrient extraction. Fed pattern motility is activated primarily by peripheral autonomic ganglia via primarily vagal inputs and also, but to a lesser extent, is controlled by the central nervous system (CNS) (see, Yoshida et al., *J. Pharmacol. Exp. Therap., 256*: 272-278 (1991); Tanaka et al., *J. Surg. Res., 53*: 588-595 (1996); Chung et al., *Can. J. Physiol. Pharmacol., 70*: 1148-1153 (1992)). Over-activation of autonomic nerves accelerates the onset and increases the duration of the fed pattern, concurrently increasing the frequency and amplitude of propagatory motor activity of the gut (Hall et al., *Am. J. Physiol., 250*: G501-G510 (1986); Johnson et al., *Am. J. Surg., 167*: 80-88 (1994), see also Examples below). As noted

above, trichothecene mycotoxins, such as DON, have been found to stimulate the fed pattern of gut motility.

"Fasting pattern" or "fasting cyclic motor pattern" of gut motor activity refers to the motor behavior of the gut in the absence of ingested food matter or before ingesting food, when no ingested material is present for propulsion from the stomach and into intestines. In the duodenum (the start of the small intestine), the fasting pattern of gut motor activity is characterized by alternating periods of spontaneous, irregular contractions and relaxations ("grouped" activity) and relatively quiescent periods ("intergroup" activity). An example of a duodenal fasting pattern with its alternating grouped and intergroup activities is shown in the early portion (between t=0 and t=30 minutes) of the recording of gut motility in Fig. 5. In the ileum (the end region of the small intestine), the fasting pattern is characterized by random contractile and/or relaxant motor activity or a generally quiescent state. Ingestion of food matter interrupts the fasting pattern of gut motility and stimulates the continuous activity of the fed pattern of gut motility.

Until recently, methods were not available for accurately measuring and characterizing gut motility in that only one component, either contraction or relaxation, could be measured under experimental conditions. More recently, however, Krantis and co-workers have developed a method of simultaneously measuring the contraction and relaxation components of gut motility for various organs of the gastrointestinal tract using miniaturized, flexible, foil strain gauges that can be attached *in vivo* to various locations on organs of the gut (see, Krantis et al., *Can. J. Physiol. Pharmacol., 74*: 894-903 (1996)). In this method, wires from the gauges attached to the organs are connected to a computerized data analysis system (see, Fig. 1). The method of Krantis et al. (1996) may be used for pharmacological, neurological, and physiological studies of

the gut using *in vivo*, *ex vivo* (organs positioned out of the body cavity), and *in vitro* (extricated tissue from gut organs) procedures (see, Examples, below). The ability to simultaneously record contractions and relaxations in gut organs and at multiple sites within an organ provides a more precise characterization of gut motility, including distinct patterns of gut motility, and the effect of food and various chemical compounds on such patterns.

In the fasted state, the gut exhibits a cyclic motor behavior known as "MMC", "migrating motor complex", or "migrating myoelectric complex". MMCs are associated with interdigestive propulsion of intestinal contents and involve sequential activation of excitatory and inhibitory neurons to propagate cycles of contractions and relaxations that originate in the stomach and terminate at the ileum. An MMC cycle consists of three distinct phases: phase I is a quiescent phase; phase II is a period of irregular spiking of activity, and phase III is a short period of rapid spike bursts of activity. MMCs provide a basic intrinsic motor pattern, which functions as a "house keeper" of the small intestine. For example, the highly propulsive phase III motor activity of each MMC cycle sweeps the intestinal lumen, clearing it of remnants to prevent bacterial overgrowth, back flow, and the accumulation of intestinal secretions (Caenepeel et al., *Dig. Dis. Sci., 34*: 1180-1184 (1989)). Using the method of Krantis et al. (1996), it is now clear that gut motility may comprise both contractions and relaxations of smooth muscle. In the absence of food, the "grouped" activity of the fasting pattern of intestinal gut motility appears to correspond to the same type of motor activity classically ascribed to phase III of MMCs. The presence of food in the intestinal lumen induces a switch from the fasting pattern to the fed pattern of gut motor activity.

The method of Krantis et al. (1996) has also enabled the discovery of the mode of action of compounds called trichothecenes or trichothecene mycotoxins on gut motility. As shown in

Examples 1 and 2, the trichothecene 4-deoxynivalenol (DON) acts at site outside the gut to stimulate the fed pattern of gut motility, which characteristically occurs after ingesting food and which signals satiety, that is, the sensation of fullness. These findings provide an explanation to the well-known anorectic or feed refusal behavior of humans and other animals that have ingested crops contaminated with fungal species that produce DON or other trichothecenes. This invention provides a method of treating obesity that takes advantage of the ability of trichothecene compounds to induce the fed pattern of gut motility and satiety. Methods of treating obesity described herein comprise administering a trichothecene or similar acting compound, which stimulates the fed pattern of gut motility and, thereby, satiety. Sensing fullness, the individual is thus given a signal to stop eating. When circulating levels of the administered compound decrease, satiety will decline and the individual may continue to eat or feed.

This invention also provides methods of regulating food intake by administering an agonist or antagonist of the P_{2X1} purine receptor (purinoceptor), which mediates grouped relaxations of gut tissue. According to the invention, a trichothecene, such as DON, which stimulates the fed pattern of gut motility, actually acts at a site outside the gut. From that remote site of action, a signal travels down neural pathways to smooth muscle cells of the gut that express P_{2X1} purine receptors, which are involved in regulating the fed pattern of gut motility (see, Fig. 2). Thus, a compound that binds and affects the P_{2X1} purinoceptor is acting at the terminal portion of the neural pathway, whereas DON or other such trichothecenes act upstream. According to the invention, one group of compounds useful in the methods described herein consists of analogs of adenosine triphosphate (ATP) which may act as agonists or as antagonists of the P_{2X1} purinoceptor. As explained below, certain types of agonists of the P_{2X1} purinoceptor

bind the receptor and stimulate the fed pattern of gut motility. Such agonists of the P_{2X1} purinoceptor may be used in lieu of trichothecenes in methods of treating obesity. An antagonist of the P_{2X1} purinoceptor is a compound that binds and blocks the receptor, thereby switching off or attenuating the fed pattern. Such P_{2X1} receptor antagonists suppress fed pattern and satiety and, thus, may be used to prolong eating time and promote weight gain.

Trichothecenes Useful in the Invention

Historically, trichothecene compounds were identified as one of the toxic secondary metabolites produced by various fungi that can contaminate crops, hence the name trichothecene mycotoxins. Animals, including humans, which ingest such contaminated crops may experience a variety of pathological symptoms of mycotoxicosis, such as refusing to eat, vomiting, diarrhea, hemorrhagic lesions in internal organs, alimentary toxic aleukia (ATA), agranolocytosis, aplastic anemia, necrotic angina, inflammation of mucous membranes, refusal to eat, convulsions, sepsis, and in some cases, death (see, for example, Ueno, "Trichothecene Mycotoxins: Mycology, Chemistry, and Toxicology," in *Advances in Nutritional Research 1980, vol. 3*, pp. 301-353 (1980)).

As used herein, "trichothecene mycotoxin" or "trichothecene" refers to a member of a group of sesquiterpenoid family of chemical compounds based on the non-olefinic parent or core compound trichothecane. All trichothecenes are modified sesquiterpenes, and contain an olefinic (double) bond (hence, trichothec*ene*) between carbon atoms at positions 9 and 10 (C-9, C-10), and an epoxy ring formed between carbon atoms at positions 12 and 13 (C-12, C-13). Thus, trichothecenes are also characterized as 12,13-epoxytrichothecene compounds. Ueno classified naturally-occurring trichothecene mycotoxins into four groups based on structural and also fungal characteristics (see, for example, Ueno, 1980). According to this classification scheme,

members of a group of trichothecenes represented by nivalenol are non-macrocyclic compounds that have the carbon-8 (C-8) substituted with a ketone (oxo-) group. In addition to nivalenol, the group of "nivalenol-related" trichothecenes include such naturally-occurring trichothecene mycotoxins as 4-deoxynivalenol (DON), trichothecolon, trichothecin, 3-acetydeoxynivalenol, 7-acetyldeosynivalenol, 3,15-diacetyldeoxynivalenol, 4-acetylnivalenol(fusarenon-X), and 4,15-diacetylnivalenol. As used herein, "DON", "4-DON", "deoxynivalenol", "4-deoxynivalenol", and "vomitoxin" all refer to the same trichothecene compound having the chemical structure shown in Fig. 3. Thus, nivalenol differs from DON in that nivalenol contains a hydroxyl group at C-4, whereas DON lacks the hydroxyl group ("4-deoxy") at position 4.

Although clearly capable of causing severe and widespread incidences of toxicosis when ingested in sufficiently high quantities, DON is nevertheless considered as one of the least potent trichothecenes with respect to sub-lethal toxicosis (see, for example, Prelusky et al., *Arch. Environ. Contam. Toxicol., 22*: 36-40 (1992); Friend et al., *Can. J. Anim. Sci., 66*: 765-775 (1986); Ueno, in Developments in Food Science IV. Trichothecenes. chemical. biological. and toxicological aspects, (Elsevier, Amsterdam, 1983) pp. 135-146).

DON is also non-mutagenic as determined using a hepatocyte-mediated mutation assay with V79 Chinese hamster lung cells (Rogers and Heroux-Metcalf, *Cancer Lett., 20*: 29-35 (1983)) or a skin tumorigenesis Sencar mouse model (Lambert et al., *Food Chem. Toxicol., 33*: 217-222 (1995)). The cellular toxicity is not mediated by alteration in deoxyribonucleic acid (DNA) synthesis or repair (Bradlaw et al., *Food Chem. Toxicol., 23*: 1063-1067 (1985); Robbana-Barnat et al., *Toxicology, 48*: 155-166 (1988)).

DON has been used in rats and chickens at doses that reduced feeding without apparent disturbance to the central nervous system (CNS) and without the undesirable clinical signs of

toxicosis (see, for example, Fitzpatrick et al., *Toxicolgy Lett., 40*: 241-245 (1988)). Furthermore, DON appears to undergo no extensive liver metabolism and is readily and predominantly eliminated in the urine. The derivatives deoxynivalenol glucuranide and de-epoxide DON have also been found in urine, apparently as the result of metabolism by microbes in the gut of animals that have received DON (see, for example, Worrell et al., *Xenobiotica, 19*: 25-32 (1989); Lake et al., *Food Chem. Toxicol., 25*: 589-592 (1987)). Accordingly, DON and the other nivalenol-related trichothecene compounds are particularly well suited for use in the methods described herein for treating obesity. Trichothecenes that are not structurally related to DON and the other nivalenol-related compounds, may also be used in methods of treating obesity provided they also stimulate the fed pattern of gut motility and at doses that do not result in any of the undesirable or severe symptoms of clinical mycotoxicosis.

Trichothecenes useful in the invention may be produced biologically from fungal cultures or by chemical synthesis. DON is an abundant, natural contaminant of corn and wheat. Thus, DON and other tricothecenes may also be isolated from contaminated crops. Alternatively, they can be isolated from the Brazilian shrubs *Baccharis magapotomica* and *cordfolia* (Kupchan et al., *J. Org. Chem., 42*: 4221-4225 (1977)).

A variety of soil fungi that have been found contaminating and growing on cereal grains and other crops produce trichothecenes as secondary metabolites. Such fungi include species of *Fusarium*, *Tricothecium*, *Trichoderma*, *Myrothecium*, *Cylindrocarpon*, and *Stachybotrys* (see, Ueno, 1980). Accordingly, trichothecenes useful in the methods described herein may be produced and extracted from fungal cultures using standard culture and production techniques (see, for example, Ehrlich et al., *Biochim. Biophys. Acta, 932*: 206–213 (1987); Ueno, 1980 and references therein).

In addition, fungi that produce trichothecenes may also be used to modify pre-existing trichothecenes. Such bio-transformation of DON and its derivatives has been undertaken in a variety of laboratories employing bacteria (Shima et al., *Appl. Environ. Microbiol., 63*: 3825–3830 (1997)) or strains of *Fusarium*. For example, *F. roseum* maintained in peptone-supplemented medium converts 3-acetyldeoxynivalenol to DON (Yoshizawa et al., *Appl. Microbiol., 29*: 54-58 (1975)). *F. nivale* can acetylate DON at the carbon at position 3 to give 3-AcDON. Furthermore, these strains can deacetylate 7,15-diacetyl-DON to give 7-AcDON.

The chemistry of the tricothecenes is well known so that various trichothecene compounds may be synthesized by chemical or biochemical procedures. The tricothecenes are sesquiterpene alcohols or esters chemically related by the tetra-cyclic 12,13-epoxytricothec-9-ene skeleton (Williams, *Arch. Environ Contam. Toxicol., 18*: 374-387 (1989)). It has been reported that certain trichothecenes related to 4-DON can also be prepared using the trichothecene T-2 toxin (4ß,15-diacetoxy-3α-hydroxy-8α-[3-methylbutyryloxy]-12,13 epoxytrichothec-9-ene) as starting material, since it is produced abundantly by *F. tricinctum* and is easy to modify at the C-3 and C-8 positions (Ehrlich et al., *Appl. Environ. Microbiol., 50*: 914-919 (1985); Udell et al., *Z. Naturfarsch, 44*: 660-668 (1989)). Removal of the C-3 hydroxyl of T-2 involves initial conversion of T-2 to the 3-phenylthionocarbonate and then reduction of this intermediate with tri-n-butyltin hydride to give 3-deoxy-T-2. This approach has been used by others to prepare 3-deoxyanguidine and 4-deoxyverrucarol (Schuda et al., *J. Nat. Prod., 47*: 514-519 (1984)).

It has also been shown that to generate the C-8 oxo-functionality (that is, trichothecenes related to DON), T-2 and deoxy-T-2 are oxidized with selenium dioxide (Bamburg et al., *Tetrahedron, 24*: 3329-3336 (1968)). Additional derivatives, such as THP-7-DON

(tetrahydropyranyl-7-DON) and DIDON (3, 7-dideoxynivalenol), have been produced by introducing a C-8 ketone into the T-2 toxin (Bamburg et al., 1968). This is achieved by first preparing 3-THP-T-2 triol and 3-deoxy-T-2 triol and then oxidizing them with manganese dioxide (MnO_2) (Warpehoski et al., *J. Org. Chem., 47*: 2897-2900 (1982)). Oxidation is not possible for preparation of 7-DON from T-2 tetraol because of competing ring cleavage reactions and the low solubility of T-2 tetraol in methylene chloride, which is used as the reaction solvent. For preparation of THP-7-DON from THP-T-2 triol, MnO_2 oxidation is the only possible method, since the acetic acid used as the solvent for selenium dioxide oxidation, would remove the tetrahydropyranyl group. The side products in the MnO_2 oxidations are trichothecene, with 15-carboxaldehyde functionality.

Identification of DON-related compounds

DON-related trichothecene compounds can be determined from mass spectra, NMR (nuclear magnetic resonance) spectroscopy, infra-red spectroscopy, anisaldehyde staining, and TLC (thin layer chromatography).

All DON-related trichothecenes should have NMR spectra showing the expected AB coupling pattern from the C-13a and C-13b protons, and a proton at 6.5 ppm for C-10 (Cole and Cox, Handbook of Toxic Fungal Metabolites (Academic Press, New York, 1981), pp. 152-263).

With anisaldehyde staining, the 8-oxo-substituted (keto) trichothecenes form a lemon-yellow adduct, whereas compounds lacking the keto group at position 8 form red or brown colored adduct.

In the infrared spectra, the carbonyl group at position 8 absorbs at 1660 – 1680 cm^{-1}. This confirms that the trichothecene retains the alpha, beta-unsaturated ketone functionality.

The mass spectral data for the acetylated analogs of DON should show the parent ion and fragment ions anticipated by loss of acetyl or acetic acid during the process.

Each trichothecene used in the methods described herein is preferably purified until it migrates as a discrete spot on thin layer chromatography (TLC). Homogeneity can be further assessed by using high pressure liquid chromatography (HPLC) in which the particular trichothecene should elute as a single peak. GC-MS (gas chromatography and mass spectroscopy) analysis has also been useful in assessing purity, for example, as in showing a single peak for each type of species of purified acetylated trichothecene (Cole and Cox, 1981).

Trichothecene Derivatives and Analogs

DON may be used in the methods of the invention, such as treating obesity, but must be used at a dose that stimulates the fed pattern of gut motility without causing any of the other undesirable side effects, including emesis (vomiting), one of the clinical symptoms of mycotoxicosis. Although the pharmaceutically acceptable dose of a trichothecene such as DON can be determined using standard methods, it would be desirable to produce a modification in a trichothecene chemical structure to yield a structurally-related compound, which is even more benign with respect to possible untoward side effects. Such a "benign trichothecene" (for example, a "benign DON") is a derivative of the original trichothecene and is expected to be comparable or more potent than the original trichothecene in stimulating the fed pattern of gut motility, but with fewer or no untoward side effects. Hence, such a derived trichothecene will exhibit one or more improved properties and will be preferred over a known trichothecene, such as DON, in methods of treating obesity.

For example, various structural features of DON provide sites on the compound that are particularly attractive candidates for modification to form DON derivatives. Advantageously,

DON is a relatively small molecule and has a limited number of sites available for modification to alter activity of this compound. Such sites include the unsaturated bond between C-9 and C-10, the presence of the 12,13 epoxy ring, the presence of hydroxyl or other goups on the structural nucleus of the trichothecene, and the occurrence of hydroxyl or other substituents at C-3, C-4, and C-15 (see, Fig. 3). In addition, space-filling molecular models reveal several features of the trichothecene nucleus that provide additional information in considering which site(s) to modify in provide a useful derivative of DON. Oxygen substituents in the A-ring (C-8 keto and C-7 hydroxvl groups) make this side of the molecule more hydrophilic than when no substituent is present or when, like in T-2 toxin, an isovaleroxy side chain is present. The presence of hydroxyl groups at appropriate positions on the nucleus modifies biological activity. For example, the difference between 4-deoxynivalenol (DON) and nivalenol is the presence of a hydroxyl group at C-4 in DON.

Studies of the relationship between trichothecene structure and the characteristic property of trichothecenes to inhibit protein synthesis have also revealed several interesting features that may be considered in making derivatives of DON or other trichothecenes that may be employed in the methods of the invention (see, Erlich et al., *Biochim. Biophys. Acta, 923*: 206-213 (1987); Rotter et al., *Env. Health, 48*: 1-34 (1996)). With respect to inhibition of protein synthesis, the most potent trichothecenes lack substitution in the A-ring containing the 8-oxo substituent, or have esterified hydroxyls. When a C-7 hydroxyl is present, hydrogen (H) bonding to the C-8 keto can occur, but this makes the ring more sterically strained. H-bonding can also occur between the C-15 and C-7 hydroxyl groups. Removal of the C-7 hydroxyl group exposes the C-15 substituent on the side of the trichothecene away from the 12,13-epoxide. In addition, the C-7 hydroxyl group must contribute to trichothecene potency since nivalenol, which possesses the

C-7 hydroxyl group, is an order of magnitude more potent than 7-DON, which lacks this hydroxyl group. It is, thus, understood that comparable or corresponding sites in DON and other trichothecenes, especially the nivalenol-related trichothecenes, may be considered as potential sites for modification to produce a derivative of DON or other trichothecene, which is useful in the methods of this invention.

However, it is also understood that an alternative to DON or other trichothecene need not be a structurally related, derivative compound, as any compound that regulates gut motility at a dose having minimal or no untoward side effects may be useful in the methods described herein.

According to the invention, a trichothecene analog is any compound which mimics one or more of the characteristic and desirable biochemical activities of a trichothecene, whether or not the compound has structural characteristics of a trichothecene (Ueno, 1980). Like DON, trichothecene analogs useful in the methods of the invention regulate gut motility by acting outside the gut, in the periphery. In particular, trichothecene analogs which are useful in the methods described herein for treating obesity, act outside the gut to stimulate the fed pattern of gut motility and, thereby, signal satiety to stop eating. Such trichothecene analogs may be structurally related or chemically derived from DON or another trichothecene (see above); an inorganic molecule; an organic molecule unrelated to the trichothecenes; biomolecules, such as nucleotides, nucleic acids, peptides, polypeptides, proteins, carbohydrates, lipids; or combinations thereof. Whether a particular compound is a trichothecene analog may be determined using one or more methods of screening for trichothecene activities described herein.

Agonists and Antagonists of the P_{2X1} Purine Receptor (Gut Neurotransmitter Receptor)

Compounds that bind the P_{2X1} subtype of purinoceptor found in gut tissue may also be used in the methods described herein. The P_{2X1} purinoceptor is a neurotransmitter receptor

present in smooth muscle of the gut and is involved in the control of gut motility (see, Examples and Fig. 2). Adenosine triphosphate (ATP) is a naturally occurring ligand of the P_{2X1} receptor. In the duodenum and ileum of the small intestine, stimulation of purinergic motor neurons releases ATP. ATP initially acts as an agonist to the P_{2X1} purinoceptor in that the first ATP molecule to bind to a P_{2X1} purinoceptor on a smooth muscle cell signals an inhibition of the smooth muscle, which then relaxes. As noted above, such relaxation is a component of gut motility that can be detected and measured using the method of Krantis et al. (1996). However, a molecule of ATP appears to remain bound to the P_{2X1} purinoceptor and thereby desensitizes the muscle to additional relaxation by ATP because other ATP molecules cannot bind the receptor to signal additional relaxation events (Smits et al., *Br. J. Pharmacol., 303*: 695-703 (1996)). Accordingly, the P_{2X1} receptor mediated pathway for gut motor activity is blocked, resulting in an observable attenuation of all gut motor activity, which cannot be influenced by additional ATP (development of tachyphylaxis). ATP is, therefore, a "desensitizing" agonist, which prevents any further relaxations, which are critical in both the fed pattern and fasting pattern of gut motility. ATP is capable of binding to all types of purinergic receptors. The synthetic ATP analog, α,β-methylene ATP, is also a desensitizing agonist, but is specific for P_{2X} species of purinoceptors. Furthermore, since the P_{2X1} subtype of purinoceptor is the P_{2X} species of receptor involved in the relaxation component of gut motility, studies using α,β-methylene ATP provide data that accurately reveal the specific neurophysiological features of relaxation in gut motility (see, Examples).

In contrast to a desensitizing agonist of the P_{2X1} receptor, such as ATP or α,β-methylene ATP, a "non-desensitizing" agonist of the P_{2X1} receptor has receptor binding properties that are necessary to provide a continual stimulation of relaxations for gut motor activity. In particular, a

non-desensitizing agonist of the P_{2X1} receptor is a compound that binds, but does not block the receptor. Each molecule of a non-desensitizing agonist is able to bind the P_{2X1} receptor, evoke a relaxation, and then dissociate to be replaced by another of its kind, which in turn signals another relaxation, and so on. Thus, non-desensitizing agonists of the P_{2X1} receptor are able to stimulate relaxation events as long as molecules of the non-desensiting agonist are available for binding to the P_{2X1} receptor. Non-desensitizing agonists of the P_{2X1} receptor stimulate the fed pattern of gut motility. Thus, non-desensitizing agonists of the P_{2X1} receptor are chemical alternatives to using DON, other trichothecenes, or trichothecene analogs in methods of treating obesity described herein.

A desensitizing agonist (see, above) or an antagonist of the P_{2X1} receptor blocks the receptor and attenuates gut motility. Such compounds may be used to inhibit the fed pattern of gut motility and, thereby, inhibit satiety. Inhibiting satiety will promote longer eating or feeding time because the feeling of fullness is not evoked. According to the invention, an antagonist or a desensitizing agonist of the P_{2X1} receptor, such as α,β-methylene ATP, is useful in methods of prolonging eating time and increasing weight gain. Such a goal is particularly useful in the meat and poultry industry where increasing weight of livestock or decreasing the time required to bring an animal to a marketable weight is commercially desirable and advantageous.

A number of structural analogs of ATP and some of their pharmacological characteristics and receptor binding properties are known (see review Harden et al., in *Annu. Rev. Pharmacol. Toxicol., 35*: 541-579 (1995)). Such compounds may serve as candidate compounds that can be further screened for the ability to bind the P_{2X1} subtype purinoceptor and affect gut motility. Alternatively, the chemical structures of such known ATP analogs may be further modified to make other ATP analogs that can then be screened for the ability to act as non-desensitizing

agonists, desensitizing agonists, or antagonists of the P_{2X1} receptor that can be used in the various methods described herein.

A class of compounds that may serve as a source of agonists or antagonists of the P_{2X1} receptor useful in this invention are anthroquinone-sulfonic acid derivatives originally described by Bohme et al. (*Chomatogr., 69*: 209-213 (1972)). Such derivatives may be viewed as ATP analogs and include a triazinyl moiety, which has been shown to antagonize certain ATP-mediated actions in the guinea pig (see, Kerr and Krantis, *Proc. Austr. Physiol. Soc., 10*: 156P (1979)). It is expected that those anthroquinone-sulfonic acid derivatives that are capable of binding the P_{2X1} receptor and regulating (that is, either stimulating or inhibiting) patterns of gut motility may be useful in various methods described herein.

Another approach for developing desensitizing agonists of the P_{2x1} receptor that are useful in treating obesity is to develop compounds from sulfonyl ureas, for example, by replacing the triphosphate moiety of the parent compound (adenosine 5'-tetrahydrogen triphosphate) with unique, innovative acidic functionalities that are known to mimic the charge distribution in diphosphate or triphosphate, but that have never before been combined with the adenosine molecule. Importantly, the adenosine-SO_2-NH-CO moiety is available for combinatorial chemistry on a polymer base (Chiron Technologies). Compounds that bind the P_{2X1} receptor and stimulate the fed pattern of gut motility are useful in methods of treating obesity according to the invention.

Screening Methods

Various methods may be employed to identify those trichothecenes, trichothecene analogs, non-desensitizing P_{2X1} receptor agonists, and P_{2X1} receptor antagonists that are useful in the methods and compositions of the invention.

Specific antibodies for detecting DON or 15-acetyl DON have been made and used in ELISA (enzyme linked immunadsorbent assay) (see, Sinha et al., *J. Agric. Food Chem., 43*: 1740-1744 (1995)). Thus, antibodies to DON and other trichothecenes may be employed in various immunological procedures, such as ELISA, to rapidly screen for derivatives or related trichothecenes that may also be employed in the methods described herein.

Structure-function relationships for a large number of 12,13-epoxytricothecenes have been determined using *in vitro* cell cultures of Vero (Green Monkey kidney) cells, murine erythroleukemia (MEL) cells, and rat spleen lymphocytes. For example, such cell culture systems were used to test the ability of various 12,13-epoxytricothecenes to inhibit peptidyltransferase activity and, hence, protein synthesis (see, for example, Erlich and Daigle, *Biochim. Biophys. Acta, 923*: 206-213 (1987); Rotter et al., *J. Toxicol. Env. Health, 48*:1-34 (1996)). In particular, tricothecenes bind to the 60S subunit of the eukaryotic ribosome and, thereby, interfere with peptidyltransferase. The degree of structural substitution on the trichothecene sesquiterpene affects the binding characteristics to the peptidyltransferase and hence the degree of inhibition of this enzyme (Erlich et al., 1987; Rotter et al., 1996).

Cell cultures as described above can be employed to test or screen compounds of unknown activity as possible candidate compounds useful in the compositions and methods of treatment described herein. Such cell-based testing and screening methods are particularly useful to test and characterize various trichothecene compounds of unknown activity, such as newly synthesized or discovered compounds having structural features of known trichothecenes, such as DON or other nivalenol-related trichothecenes. Other compounds that are not structurally related to known trichothecenes may also be screened using such cell cultures.

In cell-based screening methods, each test compound may be compared to one or more standard preparations of a known trichothecene, such as DON, which is typically prepared in stock solutions (10 μg/ml) in dimethyl sulfoxide. The concentration of dimethyl sulfoxide is adjusted so that it is always 1% (v/v) or less during incubations with the cells. Tricothecenes are generally stable for up to one year at room temperature (27°C).

Candidate compounds may be further screened and characterized using the method of Krantis et al. (1996), which uses miniaturized foil strain gauges and a computerized data analysis system to precisely and simultaneously record relaxations and contractions of smooth muscle in the gut. As noted above, the method of Krantis et al. (1996) is able to provide an actual recording of the effect of a compound on fed and fasting patterns of gut motility *in vivo*, *ex vivo*, or *in vitro* (see, Examples 1 and 2).

Binding Assays and Screens for P_{2X1} Agonists or Antagonists

Candidate compounds (also referred to as "lead" or "test" compounds) can also be tested or screened for the ability to bind or block the P_{2X1} subtype of purine receptor, which is the purinergic receptor expressed on smooth muscle and particularly involved in controlling the relaxation component of gut motility in the small intestine. Much is now known about the structure of P_2-purinoceptors (see, for example, Virginio et al., *Mol. Pharmacol., 53*:969-973 (1998); Humphrey et al., *Naunyn Schmiedeberg's Arch. Pharmacol., 352*:585-596 (1995); Bo et al., *Br. J. Pharmacol., 112*:1151-1159 (1994); and reviews in Burnstock, G., *Ciba Found. Symp., 198*: 1-28 (1996) (P_2 receptor classification) and in Surprenant, A., *Ciba Found. Symp., 198*: 208-219 (1996) (functional properties of native and cloned P_{2X} receptors)). The ability of a compound to bind the P_{2X1} purinoceptor may also be compared with that of a known receptor ligand, such as ATP or the ATP analog α, β-methylene ATP.

Successful radiolabeling of cell surface receptors for extracellular stimuli depends on the availability of ligands of high affinity, stability, and protein-binding specificity. There are no selective antagonists, and the several compounds that have been shown to competitively inhibit P_2-purinoceptors (for example, suramin, reactive blue 2) do so with only micromolar affinity and lack specificity in that they interact with many other proteins. A consistent problem has been that binding assays have been carried out under conditions, for example, with membranes, which are very different from the conditions in which biological responses to the receptor can be measured. Accordingly, direct correlations between binding constants and receptor activity constants have been difficult to make. Agonists of P_2-purinoceptors also present problems, since their binding affinities are only slightly higher than their affinities for other ATP binding proteins, and they are subject to hydrolysis by nucleotide hydrolyzing enzymes.

[^{3}H]-labeled α,β-methylene ATP has been used as a radioligand for P_{2X}-purinoceptors in preparations of urinary bladder and vas deferens smooth muscle. Generally, agonist binding affinities follow those observed in intact tissues. For example, the differences in apparent binding affinity of α,β-methylene ATP and 2-methyl-S-ATP for competition at the vas deferens binding site were only about 30-fold, and many nucleotides that are supposedly not P_{2x}- agonists also fully inhibit radioligand binding. The density of binding sites labeled with [^{3}H] α,β-methylene ATP far exceeded that observed with all other neurotransmitter receptors.

Bo et al. (*J. Biol. Chem., 267*: 17581-87 (1992)) have covalently incorporated [^{3}H] α, β-methylene ATP into a 62 kDa protein in vas deferens membranes, but the equivalence of this protein with the P_{2X}-purinoceptor has not been firmly established. [^{3}H] arylazido-aminoproprionyl-ATP also has been used in an attempt to covalently label P_{2x}-purinergic

receptors, but the equivalence of P_{2x}-purinoceptors with the 57kDa and 62kDa proteins that were labeled was not demonstrated.

Enteric smooth muscle expressing P_{2x1} receptors can be dissociated, and the isolated smooth muscle cells maintained in primary culture. These cultures can be used in a binding assay for lead or candidate compounds that are able to act as agonists or antagonists of the P_{2x1} receptor. Alternatively, embryonic kidney 293 cells which express the P_{2x1} receptors may be used (Virginio et al, *Mol. Pharmacol., 53*:969-973 (1998)). This receptor subtype is also expressed in platelets and megakaryoblastic cell lines (Vial et al., *Thromb. Haemost., 78*: 1500-1504 (1997)), as well as in HL60 cells (Buell et al, *Blood, 87*: 2659-2664 (1996)). Accordingly, any cell, including recombinantly modified cells, that expresses the P_{2X1} receptors in culture may be useful in screens for agonists or antagonists of the P_{2X1} receptor.

The P_{2X1} purine receptor has been purified (Valera et al., *Nature, 371*: 516-519 (1994)) and cloned (Sun et al., *J. Biol. Chem., 273*: 11544-11547 (1998)). The binding characteristics for recombinant P_{2x1} receptors have been described (Michel et al., *Br. J. Pharmacol., 118*:1806-1812 (1996)). A purified P_{2X1} receptor may be attached by any of a variety of linking agents to a solid substrate, such as the surface of a well in a microtiter plate, a resin particle, or the surface of an assay chip. Such arrangements allow very small quantities of compounds to be tested for the ability to bind to the receptor. Furthermore, the robotic technology that is available for screening samples in microtiter plates and assay chips permits hundreds or thousands of compounds to be accurately and continuously screened in hours with minimal supervision by the skilled practitioner.

Lead or candidate compounds identified as having an activity in one of the above screening methods can be further evaluated using an *in vitro* assay, *ex vivo* gut organ assay,

and/or an *in vivo* assay for gut motility, for example, by the method of Krantis et al. (1996) (see, Example 1). In an *in vitro* gut organ bath assay, portions of a gut organ, for example, segments of the duodenum, jejunum, and ileum of the small intestine, are excised from an animal and placed in a physiological maintenance medium, such as Krebs solution at physiological body temperature. Individual gut segments are usually mounted to record circular muscle activity, preferably at two attachment points. A compound may be injected, mixed, or applied to the extricated gut organ segments, and the effect on the organ's motility measured. In an *ex vivo* gut organ assay, the gut organs of an anesthetized animal are exposed, but maintained intact and at physiological conditions. A test or lead compound may then be conveniently applied (topically) directly on the organ, and the effect on the organ's motility monitored. In an *in vivo* assay, a compound may be injected into or ingested by an animal, and the effect on gut motility measured directly.

Sources of compounds to be tested or screened for use in the compositions and methods described herein include, without limitation, combinatorial libraries, growth media or cell extracts from fungal, bacterial, and various eukaryotic cell cultures or fermentations, and biological fluids, tissues, and serum samples from humans and other animals.

Methods of Treatment, Pharmaceutical Compositions, Modes of Administration

The pharmaceutical compositions of this invention are used in methods of treating obesity. Other compositions of this invention are formulated for administering to animals to promote weight gain, which is especially useful in raising commercial livestock for market. Humans and other vertebrate animals have the same basic gut neurophysiology with respect to controlling gut motility. Accordingly, animals that can be treated using the methods described herein include, without limitation, primates, swine, cattle, sheep, poultry and other birds, horses,

cats, dogs, and rodents, including hamsters, guinea pigs, rats, and mice. Both pharmaceutical compositions and compositions for administration to livestock described herein contain an effective amount of a compound to achieve the desired effect on gut motility without significant or undesirable side effects.

According to the invention, obesity is treated by administering to a human or other animal an effective amount of DON or other trichothecene, trichothecene analog, or non-desensitizing agonist of the P_{2X1} purinoceptor to stimulate or activate the fed pattern of gut motility and, thereby, signal satiety. In contrast, a P_{2X1} purinoceptor antagonist or desensitizing agonist, such as α,β-methylene ATP, is administered to a human or other animal to inhibit the fed pattern or stimulate the fasting pattern of gut motility and, thereby, prolong eating time.

Such compositions may be in any of a variety of forms particularly suited for the intended mode of administration, including solid, semi-solid or liquid dosage forms, for example, tablets, lozenges, pills, capsules, powders, suppositories, liquids, powders, aqueous or oily suspensions, syrups, elixirs, and aqueous solutions. Preferably, the pharmaceutical composition is in a unit dosage form suitable for single administration of a precise dosage, which may be a fraction or multiple of a dose which is calculated to produce the desired affect on gut motility. The compositions will include, as noted above, an effective amount of the selected compound in combination with a pharmaceutically acceptable carrier and/or buffer, and, in addition, may include other medicinal agents or pharmaceutical agents, carriers, diluents, fillers and formulation adjuvants, or combinations thereof, which are non-toxic, inert, and pharmaceutically acceptable. In liquid mixtures or preparations, a pharmaceutically acceptable buffer, such as a phosphate buffered saline may be used. By "pharmaceutically acceptable" is meant a material that is not biologically, chemically, or in any other way, incompatible with body chemistry and

metabolism and also does not adversely affect any other component that may be present in the pharmaceutical composition.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Pharmaceutically acceptable liquid compositions can, for example, be prepared by dissolving or dispersing an active compound that regulates gut motility as described herein and optimal pharmaceutical adjuvants in an excipient, such as, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, triethanolamine oleate.

Standard methods of preparing dosage forms are known, or will be apparent, to those skilled in this art (see, for example, Remington's Pharmaceutical Sciences (Martin, E.W. (ed.) latest edition Mack Publishing Co., Easton, PA). In the case of DON and other trichothecenes, a dose is prepared that does not result in emesis (vomiting). Such sub-emetic doses are readily determinable as has been demonstrated in a variety of animal studies (see, Examples 1 and 2).

The primary active ingredient of a composition of this invention is compound, which is a trichothecene, trichothecene analog, an agonist of the P_{2x1} receptor, or antagonist of the P_{2x1} receptor that affects gut motility. Trichothecenes such as DON are clearly capable of exerting their activity on gut motility when ingested. Accordingly, a preferred composition of this invention is formulated for oral administration. Such compounds may also be administered parenterally, for example, by intravenous, intramuscular, or intraperitoneal injection.

For oral administration, compositions may be formulated as fine powders or granules containing of the compound that affects gut motility and may also contain diluting, dispersing, and/or surface active agents. Compositions for oral administration may also be presented in water or in a syrup as a solution or suspension, in pills, tablets, capsules or sachets in the dry state, or in a nonaqueous solution or suspension wherein suspending agents may be included. Binders and lubricants may also be used in compositions for oral administration. Where desirable or necessary, flavoring, preserving, suspending, thickening, or emulsifying agents may be included. Tablets and granules are preferred oral administration forms, and these may be coated.

Parenteral administration, if used, is generally a method of injection. Injectable preparations can be prepared in conventional forms, either liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. For most purposes, a compound useful in regulating gut motility may be injected intravenously in a pharmaceutically acceptable buffer. However, it is within the scope of this invention that such a compound may alternatively be prepared as a bolus, which may contain a mordant for gradual release from an injection site. One approach for parenteral administration involves use of a slow release or sustained release system, such that a constant level of dosage is maintained (see, for example, U.S. Patent No. 3,710,795).

The exact, effective amount of a compound useful in regulating gut motility in the compositions and methods described herein will vary from subject to subject, depending on the age, weight and general condition of the subject, the severity of disease or disorder being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact amount for an ideal dose applicable to all individuals. However, it is expected

that generally a trichothecene such as DON will be used or tested in a range of 0.01 – 100 mg/kg body weight. Furthermore, the useful dosage selected for a particular individual will be a sub-emetic dose, that is, a dose that does not evoke vomiting in that individual. For commercial pharmaceutical compositions, it is understood that a pharmaceutically effective and suitable amount of trichothecene, trichothecene analog, P_{2X1} receptor agonist, or P_{2X1} receptor antagonist will be determined, in the case of human use, by the healthcare professional in studies acceptable to the standards of the United States Food and Drug Administration (or comparable agency). For use in other animals, an appropriate composition will be determined and formulated according to the standards and practices for commercial livestock feed or veterinary medicine.

Additional embodiments and features of the invention will be apparent from the following non-limiting examples.

Examples

Example 1. The following example shows that DON acts at sites outside the gut and interferes with specific intrinsic neural pathways of the stomach and small intestine, giving rise to altered patterns of motor activity. These findings show that DON induces loss of appetite (as illustrated by feed refusal in animals), and support a method of treating such loss of appetite.

Normal gastrointestinal motility is dependent on the intrinsic (enteric) neural networks of the gut wall, with modulatory inputs from the periphery and central nervous system (CNS). The intrinsic circuitry coordinates reflex activity, such as the peristaltic reflex, or complex patterned motor activity, such as interdigestive migrating myoelectric complexes (MMCs) that occur within the fasting pattern and segmentation driving fed pattern. This study evaluates the effects of the trichothecene DON on the pathways controlling interdigestive spontaneous motor activity of the rat stomach and intestine. Even though rats do not posses an emetic reflex, they do exhibit

the sickness and discomfort associated with vomiting (Andrews,P.L.R., *Br. J. Anaesth.*, 69: 2s-19s (1992), Rapley, W.A. and Hirst, M., Abstract in *Lab. Anim. Sci.*, 38: 504 (1988)). The effects of low-threshold levels of DON on the interdigestive motor pattern *in vivo* were examined. Additional information was obtained from *in vitro* gut bath experiments to obtain adjunct pharmacological information.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats (Charles River) weighing 250-350 grams. All experimental protocols were carried out according to the guidelines of the Animal Care Committee at the University of Ottawa.

In vivo Preparations.

Individual rats, fasted for 24 hours with free access to water, were anesthetized using a Halothane (4%) in oxygen mixture. Rats were maintained under Halothane (2%) anesthesia on a heated scavenging table to preserve body temperature at 37°C. The right carotid artery was exposed and cannulated using PE50 tubing to monitor blood pressure via a pressure transducer (P23ID, Gould Statham, OH) connected to an IBM PC data acquisition system. The jugular vein was cannulated (PE 50 tubing) to allow for intravenous (i.v.) injections. However, due to the short half-life of various drugs, and to evade hepatic first-pass metabolism, an intra-arterial route of administration was often necessary. In animals where drugs were to be introduced by close intra-arterial (i.a.) injections, a cannula (PE 10 tubing) was inserted from the femoral artery and fed in a retrograde direction to position the tip at the level of the superior mesenteric artery.

A median laparotomy exposed the gastrointestinal segments of interest. Foil strain gauges (Showa type N11, Durham Instruments Pickering, ON) were sequentially attached using Vet Bond (Company, City) glue to the gastric antrum (2 cm proximal to the pyloric sphincter); to

the anti-mesenteric border of the duodenum (1-2 cm distal to the gastroduodenal junction); and lateral to the anti-mesenteric border of the ileum (just proximal to the ileo-cecal junction). All foil strain gauges were oriented parallel to the longitudinal muscle layer since this affords the most sensitive setting for recording circumferential motor activity (Krantis et al., 1996). Wire leads from the foil strain gauges were exteriorized and attached to an IBM PC data acquisition system via a 3-channel interface box. The detailed method for recording and analyzing *in vivo* motility using foil strain gauges coupled to a computer based data acquisition system, has been described by Krantis et al. (1996). A schematic diagram of this method for recording gut motility is shown in Fig. 1. After completion of the surgery, the rats were turned over to the prone position, and Halothane was maintained at 1% for the remainder of the experiment.

Ex vivo Organ Preparations

The surgical procedure for these experiments was the same as described above, except rats were left in the supine position to maintain the foil gauge attachment sites exposed. This allowed for local administration of drugs directly onto the serosal surface of the gut. Regular application of warmed saline kept the exposed gut segments moist.

In vitro (Gut Organ Bath) Experiments

A separate group of rats were used for organ bath experiments after the methods of McKay and Krantis (*Can. J. Physiol. Pharmacol., 69*: 199-204 (1991)). Rats were euthanized, and 4-5 cm segments of the proximal duodenum, jejunum, and ileum were quickly removed, carefully cleared of contents, any mesenteric attachments dissected away, and then placed in an organ bath containing Krebs solution of the following composition (mM): Na^+(151.0), K^+ (4.6), Mg^{2+} (0.6), Ca^{2+} (2.8), Cl^- (134.9), HCO_3^- (24.9), $H_2PO_4^-$ (1.3), SO_4^{2-} (0.6), and glucose (7.7).

This solution was maintained at 37° C and continuously gassed with 95% O_2 : 5% CO_2 to give a pH 7.4.

Individual gut segments were then mounted horizontally to record circular muscle activity at two attachment points on the mesenteric border 25 mm apart, each opposite a frog heart clip tethering the segment to the bottom of the organ bath, and each connected to Grass isometric force transducers by thin polyester string. Mechanical activity detected by the transducers was monitored directly by a MacLab Macintosh data acquisition system (Apple Corp., Toronto, Ontario).

Each attachment point was placed under a resting tension of 1 gram and the segment allowed to equilibrate for 60 minutes before drug treatments. Organ bath preparations were washed with renewals of the bathing Krebs solution every 15 minutes and between drug challenges. Subsequent drug challenges were tested only after an equilibration period of at least 5 minutes or until the basal tone recovered to 90% of the resting tension.

Data Analysis

The motor activity recorded in the *in vivo* and *ex vivo* experiments was acquired, digitized, and stored by an IBM data acquisition system that calculated, in addition to other variables, the amplitude and frequency of motor responses (Krantis et al., 1996). Qualified responses were marked depending on their capacity to satisfy a set of six numerical parameters (separately for contraction and relaxation), based on user-defined threshold values that must be satisfied within limited time periods. These parameters were continuously monitored over sequential two minute periods and adjusted as deemed necessary to efficaciously mark motor responses with 95-100% accuracy. The data were then output and organized into tabular form for statistical analysis.

Statistical Analysis

A one-way ANOVA with a Tukey multiple analysis test was used to compare mean values, using Statgraphics Plus 5.0 software (Statistical Graphics Corp.). A probability of less than 0.05 ($p<0.05$) was regarded to be significant. All values are expressed as mean ± S.E.M of experiments.

Drugs

All the drugs utilized in the *in vivo* and *ex vivo* experiments, including DON, were dissolved in physiological saline (0.9%). The infused concentrations (delivered at a rate of 0.5 ml·min-1) were: α,β-methylene adenosine triphosphate α,β-methylene ATP, 300 mg·kg^{-1}), Nω-nitro-L-arginine methyl ester (L-NAME, 10 mg·kg^{-1}), BRL 43694 (granisetron, 80 mg·kg^{-1}), pentolinium (5x10-5 M) and hexamethonium (18 mg·kg^{-1} s.c.). All drugs were purchased from Sigma Chemical Company, Toronto, ON; except for α,β-methylene ATP, which was obtained from RBI, Natick, MA; and DON was provided by Dr. Dave Miller, Agriculture Canada, Ottawa, ON, where it was biosynthetically produced and purified according to the methods of Miller et al. (*Can. J. Microbiol., 29*: 1171-1178 (1983)) and Miller and Arnison (*Can. J. Plant Path. 8*: 147-150 (1986)).

The concentrations of drugs applied in the organ bath preparations (*in vitro*) or topically in the *ex vivo* experiments were: carbachol (0.5 mM), papaverine (10 mM), ATP (0.5 mM), DMPP (50 mM), 3-APS (0.5 mM) (Sigma Chemical Company, Toronto, ON) and DON (20 mM). Drug volumes were never more than 1% of the bath volume.

Spontaneous Motor Activity of the Gastrointestinal Tract

Under control conditions, the stomach and small intestine in anesthetized rats displayed typical spontaneous motor activity. In the gastric antrum, this consisted of oscillatory contractile and relaxant motor responses. In the proximal duodenum, spontaneous motor activity was patterned into periods of intense "grouped" activity (1-5 minute duration), comprised primarily of high amplitude, high frequency relaxations and contractions. The "grouped" activity was interposed between "intergroup" activity (3-10 minute duration), comprised primarily of low amplitude, low frequency relaxations and contractions. In contrast to the "intergroup" activity, "grouped" activity was propagatory at a rate of 3.4 ± 0.6 cm·min^{-1} in the aboral direction. Control spontaneous motor activity of the distal ileum consisted primarily of randomly occurring contractions and/or relaxations of relatively high amplitude and low frequency.

Effects of DON on Motor Activity

DON, administered systemically in a 1 or 2 mg·kg^{-1} bolus, did not affect control motor activity. However, at 10 mg·kg^{-1}, DON disrupted spontaneous motor patterns, as described below. Treatment with 20 mg·kg^{-1} of DON did not yield significantly greater effects.

Gastric Antrum

Within 2 minutes following intravenous injection, DON (10 mg·kg^{-1}, i.v., n=7) inhibited antral motor activity (Figure 4), attenuating ($p<0.05$) both spontaneous contractions (to 20 ± 6 % of control) and relaxations (to 27 ± 11 % of control). This effect was transient, as motor activity recovered to 90% of the control level within 18 ± 3 minutes. A proximate reapplication of DON was typically without effect.

Proximal Duodenum

Within 2 minutes of systemic DON (10 $mg \cdot kg^{-1}$, n=12) injection, spontaneous duodenal motor activity transformed from the control pattern of alternating "grouped" and "intergroup" activity to a period (46 ± 15 minutes) of sustained "grouped"-like activity (Fig. 5). This hyperactivity was not significantly different in amplitude or frequency from the control "grouped" motor activity. Within 60 minute following the administration of DON, the control pattern of alternating "grouped" and "intergroup" activity recovered. Subsequent injection of DON (n=6) did not significantly alter the motor pattern, indicative of the development of tachyphylaxis. This tachyphylaxis to DON was relatively short-lived, such that retesting DON (n=5) 30 minutes later, again elicited hyperactivity ($p<0.05$); however the duration of this activity (24 ± 14 min) was considerably reduced compared to the duration of the initial DON induced hyperactivity.

Distal Ileum

Systemic injection of DON (10 $mg \cdot kg^{-1}$, n=9), within 2 minutes, evoked hyperactivity of ileum. The frequency and amplitude of contractile and relaxant motor responses were significantly ($p<0.05$) increased. Thus, the pattern of gut motility in the ileum in the presence of DON resembled the characteristic fed pattern of gut motility that occurs when food has been ingested and needs to be propelled through the gut. This effect lasted 63 ± 22 minutes. Thereafter, motor activity gradually recovered to control levels. In parallel to the duodenum, tachyphylaxis to readministration of DON (n=6) also developed in the ileum. This tachyphylaxis persisted for up to 90 minutes, after which time, hyperactivity could again be induced by readministration of DON (n=6).

Effects of Locally Administered DON

Ex vivo preparations exhibited patterned motor activity similar to that of the *in vivo* preparations. Direct (topical) application of 20 mM DON (a concentration considerably greater than the *in vivo* dose) to the serosa of the gastric antrum (n=3), proximal duodenum (n=3), or distal ileum (n=3) did not evoke any comparable motor responses to those seen *in vivo*. The vitality of the gut regions examined was verified by observing predictable responses to pharmacological stimuli that are known to act directly on smooth muscle, such as, papaverine (10 mM), which relaxes smooth muscle, and carbachol (0.5 mM), which induces cholinergic-muscarinic receptor mediated contractions. Topically applied DON did not interfere with the action of these drugs.

Isolated gut bath preparations (n=5) of duodenum, jejunum, and ileum reacted with either a contraction or a relaxation to an application of carbachol (0.5 mM) or papaverine (10 mM), respectively. In addition, the gut segments exhibited relaxant responses to the putative non-andrenergic, non-cholinergic (NANC) inhibitory transmitter ATP (0.5 mM) and to neural stimulation, using the $GABA_A$ receptor agonist 3-APS (0.5 mM) or the nicotinic receptor agonist DMPP (50 mM). However, in these same preparations, DON (20 mM) was ineffective. Furthermore, DON did not interfere with the responsiveness of these gut segments to the pharmacologic agents tested.

Pharmacology of DON Induced Hyperactivity

L-NAME: In anesthetized rats exhibiting spontaneous motor activity, the nitric oxide (NO) synthase inhibitor, L-NAME, attenuates NO-mediated "intergroup" relaxations and enhances "grouped" activity of the duodenum (unpublished observations). Therefore, the effect of L-NAME on the action of DON was examined. Systemically administered L-NAME (10

mg·kg^{-1}, n=5), did not alleviate ($p>0.05$) DON induced hyperactivity of frequency and amplitude of relaxations in the duodenum (see, Figs. 6A, frequency, and 6B, amplitude). In the ileum, L-NAME always enhanced both the frequency and amplitude of relaxations of spontaneous motor activity to the same level as with the presence of DON alone (see, Figs. 6C, frequency, and 6D, amplitude).

Purinoceptor tachyphylaxis: Spontaneous duodenal "grouped" relaxations are specifically mediated through P_{2X}-receptor related purinergic transmission (unpublished observations). This was confirmed here following desensitization of the P_{2X}-purinoceptors with prolonged exposure to the specific agonist, α, β-methylene ATP. Initial injection of α,β-methylene ATP (300 mg·kg^{-1}, i.a, n=3) induced a prominent relaxation. Following recovery to baseline tone, re-challenge with α,β-methylene ATP was without effect, indicative of the development of tachyphylaxis. Under these conditions, spontaneous duodenal "grouped" relaxations and ileal relaxations were specifically blocked. In addition, DON induced hyperactivity was also abolished during α,β-methylene ATP induced tachyphylaxis in both the duodenum (n=8) and ileum (n=4).

Nicotinic receptors: Cholinergic nicotinic mechanisms are fundamentally involved in the control of intestinal motility (Furness and Costa, *Neurosci., 5*: 1-20 (1980), Gershon, *Ann. Rev. Neurosci. 4*: 227-272 (1981)). *In vivo*, treatment with the ganglionic nicotinic antagonists, pentolinium (50 mM topical application, n=2) or hexamethonium (18 mg·kg-1, s.c., n=2, not shown), significantly reduced the frequency and amplitude of the DON induced hyperactivity in the duodenum and ileum.

Granisetron: In the duodenum, systemically administered 5-HT$_3$ receptor antagonist granisetron (80 mg·kg^{-1}, n=8) attenuated ($p<0.05$) the frequency and amplitude of spontaneous contractions and relaxations of the "grouped" activity (compare broadly spaced diagonal bars

(granisetron alone) with open bars of control grouped activity in Figs. 7A - 7D). This effect of granisetron persisted for up to 30 minutes. However, since the duodenum did not exhibit desensitization to the actions of this drug, granisetron was repeatedly readministered to maintain blockade of the "grouped" activity. Under these conditions, DON (10 $mg \cdot kg^{-1}$, n=5) consistently induced ($p<0.05$) hyperactivity (compare narrowly spaced diagonal bars (DON alone) with filled bars (granisetron + DON) in Figs. 7A-7D).

Similarly in the ileum, granisetron (80 $mg \cdot kg^{-1}$, i.a.) attenuated ($p<0.05$) gut motility (see Fig. 11A), both spontaneous contractile and relaxant motor responses to 40 ± 18% (n=4) and 27 ± 10% (n=3), respectively, of control levels; however, it did not antagonize the DON induced hyperactivity.

The purpose of these experiments was to characterize motor patterns at the level of the stomach and small intestine in anesthetized rats exposed to the mycotoxin DON. Systemic injection of DON disrupted the gastric antrum oscillatory motor activity, replacing it with a quiescent pattern; and in the duodenum, DON induced a hyperactivity in place of the spontaneous cyclic pattern of propagatory and non-propagatory motor activity. DON also caused hyperactivity of the existing motor pattern in the ileum. The patterned activity induced by DON was reminiscent of a typical "fed pattern" motor activity. This study focused on the effects of exposure to low levels of DON - not emesis inducing high levels - since low level DON contamination is the primary cause of weight loss in swine. The action of DON was maximal with 10 $mg \cdot kg^{-1}$ of DON; this dose is comparable to other studies using rodents, where up to 40 $mg \cdot kg^{-1}$ (i.v.) of DON was used to induce alterations in feeding (Rapely et al., *Lab. Anim. Sci., 38*: 5041 (1988)). In fact, the natural occurrence of DON in undergrade Canadian,

American, Dutch and Korean grains is 2.2 $mg \cdot kg^{-1}$ of feed (Park et al., *Food Additives Contam., 9*: 639-645 (1992), Park et al., *Appl. Environ. Microbiol., 62*: 1642-1648 (1996), Ryu et al., *Food Additives Contam., 13*: 333-341 (1996), Veldman et al., *Food Additivies Contam., 9*: 647-655 (1992)), while in feeding studies with swine, dietary DON concentrations of up to 8.5 $mg \cdot kg^{-1}$ do not evoke emesis (Trenholm et al., *J. Am. Vet. Med. Associ., 185*: 527-531 (1984), Friend et al., *Can. J. Anim. Sci., 66*: 765-775 (1986)).

In swine, anorexia is maintained indefinitely with continuous ingestion of very high dietary DON concentrations (Foresyth et al., *App. Environ. Microbiol., 34*: 547-552 (1977), Friend et al., *Can. J. Anim. Sci., 66*: 765-775 (1986), Friend et al., *Can. J. Anim. Sci., 66*: 1075-1085 (1986), Trenholm et al., *J. Am. Vet. Med. Assoc., 185*: 527-531 (1984)). However with low levels of DON, the reduced rate of growth lasts for several days, after which time, weight gain recovers to acceptable "normal" rates (Friend et al., *Can. J. Anim. Sci., 66*: 7650775 (1986), Trenholm et al., *J. Am. Vet. Med. Assoc., 185*: 527-531 (1984)). This progressive tolerance to DON might be explained by the development of a reversible tachyphylaxis to DON, evident in the rats examined in this study. DON induced hyperactivity in the small intestine lasted up to 60 minutes, then full restoration of control motor patterns followed. Subsequently, responsiveness to routinely applied pharmacological stimuli was maintained; except for DON, which was ineffective after proximate successive applications, characteristic of the development of tachyphylaxis to DON. However, this tachyphylaxis was not sustained, possibly due to the high rate of DON detoxification in rats (Prelusky et al., *Fund. Appl. Toxicol., 10*: 276-286 (1988)). In swine, DON is efficaciously absorbed and slowly eliminated or detoxified (Friend et al., *Can. J. Anim. Sci., 66*: 765-775 (1986), Prelusky et al., *Fund. Appl. Toxicol., 10*: 276-286 (1988)), resulting in elevated levels of DON in the plasma for approximately 3 hours. However at very

high doses, DON has been detected in plasma up to 9 hours after ingestion (Coppock et al., *Am. J. Vet. Res., 46*: 169-174 (1985), Prelusky et al., *Fund. Appl. Toxicol., 10*: 276-286 (1988)). Swine are continuous feeders for much of the day, and this may explain how critical levels of DON may accumulate in the body and remain elevated for long periods of time in swine. Interestingly, necropsies and organ weight evaluation of animals exposed to mycotoxins, did not provide any indication of serious toxicity due to DON at the low concentrations tested in this study (Trenholm et al., *J. Am. Vet. Med. Assoc., 185*: 527-531 (1984)).

Many drugs, in particular emetic agents, alter intestinal motility at the level of the gut where they activate vagal afferents projecting to autonomic ganglia and/or the vomiting center of the central nervous system (CNS), which in turn reflexively stimulate the gut (Castex et al., *Brain Res., 688*: 149-160 (1995), Cubeddu et al., *Sem. Oncol., 19*: 2-13 (1992)). However, results from the *ex vivo* and *in vitro* experiments presented herein indicate that while the isolated gut segments were sensitive to a variety of pharmacological stimuli, directly administered DON was without effect. Thus, DON must exert its effects indirectly, from sites outside the gut. This finding is further supported by reports by others of a delayed time-to-onset of DON induced effects following intragastric versus intravenous injection (30 min versus 15 min, respectively) (Coppock et al., *Am. J. Vet. Res., 46*: 169-174 (1985), Foresyth et al., *Appl. Environ. Microbiol., 34*: 547-552 (1977), Prelusky et al., *Natural. Toxins, 1*: 296-302 (1993)).

Under normal circumstances, feeding interrupts the fasting cyclic motor pattern at all levels of the gut, replacing it with continuous, irregular low level activity (segmentation, fed-pattern). As mentioned above, segmentation is characterized by narrow annular contractions interposed between relaxations in the small intestine, and reduced motility in the gastric antrum (Ducrotte et al., *Nutrition, 12*: 123-124 (1996), Hall et al., *Am. J. Physiol., 250*: G501-G510

(1986)). The fed pattern functions to mix intestinal contents and delay anterograde propulsion to enhance substrate absorption (Lundgren et al., *Dig. Dis. Sci., 34*: 264-283 (1989)). Fed pattern motility is activated by peripheral autonomic ganglia via primarily vagal inputs and is controlled, to a lesser extent, by the CNS (Chung et al., *Can. J. Physiol. Pharmacol., 70*: 1148-1153 (1992), Tanaka et al., *J. Surg. Res., 53*: 588-595 (1996), Yoshida et al., *J. Pharmacol. Exp. Therap., 256*: 272-278 (1991)). Over-activation of autonomic nerves accelerates the onset and increases the duration of the fed pattern, concurrently, increasing the frequency and amplitude of propagatory motor activity (Hall et al., *Am. J. Physio., 250*: G501-G510 (1986), Johnson et al., *Am. J. Surg., 167*: 80-88 (1994)). This motor activity is similar to the DON induced hyperactivity in the small intestine, observed in these experiments. In addition, DON induced inhibition of antral motor activity and delay of gastric emptying (Fioramonti et al., *J. Pharmacol. Exp. Therap., 266*: 1255-1260 (1993)), is also characteristic of the fed pattern (Hall et al., 1986). Taken together, these results indicate that DON, from extrinsic sites, stimulates pathways mediating the fed pattern, either via peripheral autonomic ganglia or vagal efferents.

Fed pattern motility can be partially activated by the suppression of inhibitory nervous influences (Lundgren et al., 1989). Hence, DON, acting outside the gut, could stimulate hyperactivity by eliminating the tonic suppression of the enteric neural circuits controlling gut motility. NO (nitric oxide) is proposed to be a tonically released inhibitory mediator that modulates gastrointestinal motility (Daniel et al., *Am. J. Physio., 266*: G31-G39 (1994), Gustafsson et al., *J. Aut. Nerv. Sys. 44*: 179-187 (1993), Hryhorenko et al., *J. Pharmacol. Exp. Therap., 271*: 918-926 (1994)). In the *in vivo* experiments, treatment with the NO synthesis inhibitor L-NAME (10 mg/kg, i.v.) mimicked to some degree the effects of DON, by

potentiating specific motor activity of the duodenum and ileum. However, L-NAME treatment did not affect DON actions in the gut.

These experiments provide valuable insights into the pathways mediating DON effects in the gut. While the NO synthase inhibitor, L-NAME, selectively blocked spontaneous "intergroup" duodenal relaxations, and potentiated grouped motor activity, it did not affect DON induced hyperactivity. By contrast, α,β-methylene ATP induced tachyphylaxis, which selectively attenuated "grouped" relaxations, also prevented DON evoked hyperactivity in the duodenum. There is strong evidence suggesting that ATP and NO are NANC inhibitory neurotransmitters in the rat duodenum (Katsuragi et al., *J. Pharmacol. Exp. Therap., 259*: 513-518 (1991), Manzini et al., *Eur. J. Pharmacol., 123*: 229-236 (1986), Postorino et al., *J. Auton. Pharmacol., 15*: 65-71 (1995), Windschief et al., *Br. J. Pharmacol., 115*: 1509-1517 (1995)). This targeting of purinergic relaxations by DON was also apparent in the ileum, where DON induced hyperactivity was blocked by α, β-methylene ATP tachyphylaxis. Until now, the identity of the transmitter mediating spontaneous ileal relaxations *in vivo* has not been examined in the rat. However, there is a large body of *in vitro* functional evidence for both ATP and NO to mediate NANC relaxations in the rat ileum (Belai et al., *Cell. Tiss. Res., 278*: 197-200 (1994), Fargeas et al., *Gastroenterol., 102*: 157-162 (1992), Mahmod and Huddart, *Comp. Biochem. Physiol., 106C*: 79-85 (1993), Smits et al., *Br. J. Pharmacol., 118*: 695-703 (1996)).

The observation that nicotinic receptor blockade abolished both spontaneous and DON induced motor activity in the small intestine explains results from a several previous studies. Nicotinic ganglionic transmissions are known to mediate cholinergic stimulation of both excitatory and inhibitory intramural neurons that modulate and process enteric neural signals (Bornstein et al., *Clin. Exp. Pharmacol. Physiol., 21*: 441-452 (1994), Gershon, *Ann. Rev.*

Neurosci., 4: 227-272 (1981)). In fact, cholinergic neurons mediate all motility patterns of the gastrointestinal tract, including the peristaltic reflex and MMCs (Chung et al., *Am. J. Physiol., 267*: G800-G809 (1994), Gershon et al., 1981, Torsoli and Severi, *J. Physiol., 87*: 367-374 (1993)). Therefore, treatment with nicotinic antagonists would effectively block most if not all enteric neural circuits.

It has been reported that DON induced inhibition of gastric emptying and activation of duodenal phase III MMC activity in conscious rats, is blocked by 5-HT_3 receptor antagonists (Fioramonti et al., 1992). The concept of 5-HT_3 receptors mediating the effects of DON in the gut is intriguing, since 5-HT_3 receptor antagonists are well defined anti-emetic agents (Hesketh and Gandara, *J. Natl. Canc. Inst., 83*: 613-620 (1991), Perez, I J. Clin. Oncol., *13*: 1036-1043 (1995)), and have been shown to prevent emesis induced by high doses of DON (Prelusky et al., 1993). However, the dose of DON tested in this study is reasoned to be very much below the "emetic" dose.

5-HT_3 sites on vagal afferents are not likely to be involved in DON actions related to gut motor activity, since DON was ineffective when applied directly onto the exposed gut of a whole animal. 5-HT_3 receptors are also localized to myenteric neurons that are involved in the enteric circuits regulating the interdigestive motor pattern (Hoyer, *Neuropsychopharmacol., 3*: 371-383 (1990), Yoshida et al., 1991), and these neurons may occur within the enteric pathway(s) targeted by DON. Treatment with granisetron, a potent and specific 5-HT_3 receptor antagonist, at a sufficiently high dose that abolished spontaneous "grouped" activity in the duodenum and motor activity of the ileum, did not affect DON induced hyperactivity. Therefore, DON and 5-HT operate through different pathways that target common enteric elements in the small

intestine. However, both the DON activated pathway and the 5-HT_3 receptor-dependent pathway converge on the same population of inhibitory purinergic motor neurons.

These findings provide an explanation for DON induced feed refusal. The effects of "low" threshold levels of DON are manifest in the disruption of gut motility; antral motor activity is diminished, and motor activity of the small intestine is intensified. Together, these motor patterns exemplify the "fed state", a state normally associated with satiation. Under these circumstances, a human or other animal ceases to eat. This DON induced fed pattern is transient, and the interdigestive cyclic pattern soon recovers, presumably because DON plasma levels fall below threshold. By the time of the next meal or feeding, the human or other animal begins to consume the DON contaminated diet again, threshold plasma levels of DON are attained, the fed pattern is triggered, satiety is signaled and feeding stops prematurely. The cycle continues, and the shorter eating or feeding periods result in overall lower food intake. In this regard, it is interesting that in feeding trials (Braithwaite et al., *Proc. Can. Soc. Animal Sci.*, : (1995)) comparing ad libitum DON-fed pigs (low levels, 4 ppm of dietary DON) with "pair fed-fed" pigs restricted to the same amount of feed as the DON-fed animals, the DON-fed pigs resembled control "satiated" pigs in their activity and behavior. However, pair-fed pigs displayed behaviour typical of hungry pigs.

A consistent feature of this study, was the targeting by DON of enteric P_{2X}-purinoceptor mediated inhibitory enteric motor innervation. This innervation also involves nicotinic receptors, but, as described above, the ubiquitous distribution and involvement of nicotinic sites within enteric neural circuits precludes the use of nicotinic antagonists to counteract the actions of DON. More promising however, is the specificity of DON in activating P_{2X}-purinoceptor related activity. P_{2X}-purinergic sites represent a highly restricted component of enteric pathways,

and hence targeting these sites may represent a simple approach for counteracting DON effects in the gut.

Example 2. The effects of DON on spontaneous motor activity of the gastrointestinal tract in swine *in vivo*: involvement of enteric P_{2x}-purinoceptors.

This example demonstrates that the trichothecene DON affects gut motility by acting at a site in the peripheral nervous system and that the affect of DON may be counteracted by the P_{2X1} purinoceptor desensitizing agonist, α,β-methylene ATP, which binds with high affinity to the P_{2X1} purinoceptor on gut tissue. The intense binding by this purinergic ATP analog not only desensitizes the P_{2X1} purinoceptor regulation of gut motility from DON, but also can shut down the pathway of regulation of gut motility effectively as an P_{2X1} purinoceptor antagonist.

Surgical Preparation

Male Yorkshire pigs (10-15 kg live weight) weaned for one week were fasted for 12 hours overnight, with free access to water. On the morning of the surgery, animals were sedated using Ketamine (8 $mg \cdot kg^{-1}$) via an intramuscular injection. Ketamine is a dissociative anaesthetic, which causes an increase in blood pressure and skeletal tone, and the trachea will be stiff. A cataleptic sedation is produced with a lack of awareness of the surroundings. However, salivary secretions are increased and hence airway obstruction is a hazard; yet atropine cannot be used. Anesthesia was induced using a halothane-oxygen mixture by application of a face mask. Topical pharyngeal anaesthesia was provided using 1-2 doses of lidocaine aerosol (10 mg per dose, Xylocain, Sigma). Animals were then intubated, and a surgical plane of anesthesia was achieved using halothane (3-4%) in oxygen (200 $ml \cdot min^{-1}$) via a closed non-rebreathing circuit. A catheter was inserted into a superficial ear vein for electrolyte replacement (0.9% saline) and

intra venous drug injections. The femoral artery was also cannulated for intra-arterial drug injections. PE 205 tubing was fed in a retrograde direction such to place the tip of the cannula at the level of the superior mesenteric artery. Blood pressure was also monitored through this arterial catheter by way of a pressure transducer (P23ID, Gould Statham, OH, USA) connected to an online IBM data acquisition system. The animals were then subjected to a laparotomy, and foil strain gauges (Showa type N11, Durham Instruments, Pickering, ON) were affixed, using Vet Bond glue (after the methods of Krantis et al., 1996), onto the serosa of the gastrointestinal tract. One strain gauge was placed on the gastric antrum (5-10 cms distal to the pylorus); a second gauge was placed on the anti-mesenteric border of the proximal duodenum (2-10 cms from the pylorus), and a final gauge was attached onto the serosa of the distal ileum (2-10 cms distal to the cecum). All three foil strain gauges were oriented parallel to the axis of the longitudinal muscle. Leads from the strain gauges were exteriorized and attached to the IBM data acquisition system, via an interface box. Following completion of the surgery, the pigs were turned over to their side, and a light plane of anesthesia was maintained for the remainder of the experiment by 1-2% halothane.

Data Analysis

Motor activity was continuously recorded from all foil strain gauges simultaneously using a data acquisition software (AD1000 analog to digital conversion card, Real Time Devices Inc., Dr. Frank Johnson, Institute of Medical Engineering, University of Ottawa) and an IBM compatible computer. Qualified motor responses were selected based on their capacity to satisfy two sets (for contractions and relaxations) of six numerical values. These values defined threshold duration and magnitude parameters that efficaciously marked motor activity based on the user's visual inspection of the recordings. The user is able to continuously monitor these

parameters over sequential two minute periods, and adjust the values as deemed necessary to efficaciously mark motor responses within 95-100 % accuracy. To quantify the recordings, the data acquisition software outputs the frequency, amplitude, area, time to peak and duration of both contractile and relaxant motor responses. A description of the recording and analysis method is detailed in Krantis et al., 1996.

Statistical Analysis

A one-way ANOVA with a Tukey multiple analysis was used for comparison between mean values, using Statgraphics Plus 5.0 program. A probability of less than 0.05 ($p<0.05$) was regarded to be significant. All values are expressed as mean ± S.E.M. of experiments.

Drugs

All the drugs utilized in the *in vivo* and *ex vivo* experiments, including DON, were dissolved in physiological saline (0.9%). The infused concentrations (delivered at a rate of 0.5 $ml \cdot min^{-1}$) were: α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$), L-NAME (10 $mg \cdot kg^{-1}$), granisetron (80 $\mu g \cdot kg^{-1}$), pentolinium ($5x10^{-5}$ M) and hexamethonium (18 $mg \cdot kg^{-1}$ s.c.). All drugs were purchased from Sigma Chemical Company, Toronto, ON; except for α,β-methylene ATP and methylthio ATP, which were obtained from RBI, Natick, MA; and DON was provided by Dr. Dave Miller, Agriculture Canada, Ottawa, ON, where it was biosynthetically produced and purified according to the methods of Miller and Arnison, 1986.

Spontaneous Motor Activity of the Gastrointestinal Tract

Stomach: Motor activity in the gastric antrum generally consisted of oscillatory contractions and relaxations. These were either present for the entire duration of the control

recording or they occurred randomly. A motor pattern analogous to MMCs was not evident in the stomach recordings. A summary of the spontaneous motor activity is shown in Table 1.

Duodenum: Spontaneous motor activity consisted of an irregular pattern of contractile and/or relaxant motor activity (Table 1). Occasionally, activity reminiscent of MMCs, consisting of phase III propagatory type activity ("grouped" activity) and quiescent periods, were evident. The duration of the "grouped" activity was approximately 5 minutes, however the cycle length could not be accurately determined, since the "grouped" activity did not arise more than 2 or 3 times during the control period, which only lasted up to 2 hrs in our experiments. Indeed, MMCs in fasted pigs are known to have a cycle length of 70-115 minutes. In our experiments, the "grouped" activity consisted of relatively high amplitude, high frequency relaxations and contractions: frequency of contractions: 11.9 ± 0.5 events·min^{-1}; amplitude of contractions: 0.08 $\pm$ 0.01 g; frequency of relaxations: 12.9 ± 0.8 events·min^{-1}; amplitude of relaxations: 0.07 ± 0.01 g.

Ileum: The ileum usually exhibited random contractile and/or relaxant motor activity (Table 1). MMC-like activity was rarely observed. In one third (n=5) of the experiments, motor activity of the ileum was in a quiescent state; however in these experiments, the ileum proved to be responsive to DON treatment.

TABLE 1. Characteristics of spontaneous interdigestive motor activities in anesthetized pigs

Parameters	Stomach	Duodenum	Ileum
Amp. contraction	0.16±0.04	0.07±0.01	0.05±0.01
Amp. relaxation	0.19±0.03	0.07±0.01	0.05±0.01
Freq. contraction	4.8±0.5	6.7±0.6	3.2±0.5
Freq. relaxation	5.5±0.3	6.8±0.5	3.6±0.5

Values represent the mean±SEM of data obtained from 12 pigs.
Amp: Amplitude (grams tension); Freq: Frequency (events/min.); Dur: Duration (seconds).

Effects of DON in the Stomach

DON was administered at 0.1 $mg \cdot kg^{-1}$ (n=3), 0.7 $mg \cdot kg^{-1}$ (n=2) and 1.0 $mg \cdot kg^{-1}$ (n=10), via either an intravenous or intra arterial route. Within 5 minutes following injection, DON (n=6) decreased ($p<0.05$) the frequency and amplitude of the spontaneous contractile and relaxant motor responses. The duration of this DON induced inhibition lasted from 10 minutes up to an indefinite period of time. By contrast, in three experiments, DON increased ($p<0.05$) the frequency and amplitude of the spontaneous motor activity by 182 ± 40 % and 206 ± 38 % respectively; this effect lasted up to 30 minutes before recovering back to the control pattern. This differential action of DON was not obviously correlated to either the dose injected or the route of administration. Furthermore, neither the dose injected nor the route of administration induced any changes to the mean arterial blood pressure, which continued a steady level for the duration of the control and DON treatment periods.

Effects of DON in the Duodenum

The effects of DON were more consistent in the duodenum (n=21), where it always potentiated ($p<0.05$) the spontaneous motor activity. A systemically administered dose of DON greater than or equal to 1 $mg \cdot kg^{-1}$ consistently induced significant potentiation; it also represents the dose which increased the frequency, as well as the amplitude, of duodenal motor activity. The frequency of the DON induced hyperactivity typically remained elevated for the entire duration of the experiment, while the amplitude of the motor responses progressively recovered to control levels. Dose effects of DON on frequency and amplitude parameters of contractile and relaxant motor activity were examined for three dosages: 0.1 $mg \cdot kg^{-1}$, n=3; 0.7 $mg \cdot kg^{-1}$, n=3; 1.0 $mg \cdot kg^{-1}$, n=12; 10 $mg \cdot kg^{-1}$, n=3. A clear dose-response effect was evident only for the amplitude of the motor activity. In addition, enhanced motor activity due to DON at 10 $mg \cdot kg^{-1}$ was not

significantly different from the effects of DON at 1 $mg \cdot kg^{-1}$.

Effects of DON in the Ileum

Systemically administered DON induced a dose-dependent increase in the ileal motor activity. DON was given at doses of 0.1 $mg \cdot kg^{-1}$ (n=3); 0.7 $mg \cdot kg^{-1}$ (n=3); 1.0 $mg \cdot kg^{-1}$ (n=12); and 10 $mg \cdot kg^{-1}$ (n=4). In the ileum, the dose-response effect was evident in both frequency and amplitude parameters of the motor activity. However, maximal effects of DON occurred at a dose equal to or greater than 1 $mg \cdot kg^{-1}$, where both the frequency, as well as the amplitude, of the contractile and relaxant spontaneous motor activity were significantly ($p<0.05$) increased. Thirty to sixty minutes following the DON enhanced activity, the frequency and amplitude of the present motor activity started to decreased, however, two hours after the initial injection of DON, the motor activity was still significantly higher than control.

Effects of α,β-methylene ATP against DON Induced Hyperactivity

α,β-methylene ATP was always administered during the DON induced hyperactivity. This afforded an internal control for DON action. At the dose of 300 $\mu g \cdot kg^{-1}$, α,β-methylene ATP, given intra-arterially, induced only a transient (less than 1 minute) increase in the mean arterial blood pressure.

Administration of α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a.) always induced an initial relaxant response in the stomach, however, it did not counteract the effects of DON on gastric motor activity.

Upon injection, α,β-methylene ATP (175 $\mu g \cdot kg^{-1}$, i.a.) usually induced a small phasic relaxation of the duodenum. However, DON induced hyperactivity did not appear to be affected. A higher dose of α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a.) more consistently induced an initial phasic relaxation (0.5 ± 0.1 g, n=10), with subsequent transient (3-10 minutes) reduction in the

DON induced hyperactivity. α,β-methylene ATP significantly decreased the amplitude, but not the frequency, of the DON induced relaxations and contractions (see, Fig. 8). When α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, n=3) was readministered 10-20 minutes following the initial injection, the duodenum again relaxed. However, there appeared to be no further effect on the DON induced hyperactivity.

Analogous results were observed in the ileum, where α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a.) induced a large phasic relaxation (1.2 ± 0.2 g, n=6) upon injection, and reduced DON induced hyperactivity. The efficacy of α,β-methylene ATP in reducing the amplitude, as well as the frequency, of DON induced relaxations and contractions in the ileum is presented in Fig. 9. In three experiments, α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$) was readministered within 10 minutes of the initial dose, to test for the development of tachyphylaxis. The amplitude of the relaxation to α,β-methylene ATP was reduced by 68 ± 18% compared to the initial administration of this agent.

Example 3. Non-adrenergic, non-cholinergic (NANC) control of interdigestive motor activity in the rat small intestine, *in vivo*.

This example provides a study of the neural pathways controlling interdigestive motor activity in different regions of the rat small intestine *in vivo*. The data in this example, along with the data in Examples 1 and 2, above, support the neural circuitry modulating gut motility shown in the schematic representations of Figs. 2 and 10.

The migrating motor complex (MMC) is associated with interdigestive propulsion of intestinal contents, and like peristalsis, involves sequential activation of excitatory and inhibitory pathways. The neural circuitry underlying peristalsis comprises excitatory (primarily

cholinergic) and inhibitory non-adrenergic, non-cholinergic (NANC) motor neurons innervating the gastrointestinal smooth muscle, as well as excitatory and inhibitory interneurones (Costa and Brookes, *Am. Gastroenterol., 89*: S129-S137 (1994)). However, little is known about the intramural neurons controlling interdigestive motility, mainly since MMCs are not easily assessed *in vitro*. Moreover, analysis of MMCs *in vivo* has for the most part focussed on the contractile activity only. *In vivo* motility studies in this laboratory utilizing a new method based upon miniaturized foil strain gauges affixed to the serosa of the gut, revealed that propagatory intestinal motor activity, characteristic of MMCs, consist of contractions as well as relaxations (Krantis et al., 1996). Spontaneous interdigestive relaxations occurring within the propagatory motor activity (MMCs) can be distinguished from the non-propagatory relaxations based upon their dependence on ATP and nitric oxide, respectively (Glasgow et al., *Am. J. Physiol, 276 (Gastrointest. Liver Physiol., 38)*: G889-G896 (1998)). Nitric oxide (NO) is an inhibitory transmitter (Postorino et al., *J. Auton. Pharmacol., 15*: 65-71 (1995), Smits et al., *Br. J. Pharmacol., 118*: 695-703 (1996)) and has been implicated in the regulation of MMCs and postprandial motility (Hellstrom and Ljung, *Neurogastroenterol. Motil., 8*: 299-306 (1996), Sarna et al., *Am. J. Physiol., 265*: G759-G766 (1993)). However, it is not clear whether NO functions independently or interacts with other transmitters in a neuromodulatory fashion. A number of recent studies suggest a prominent interplay between NO and VIP in the regulation of neuromuscular activity (Daniel et al., *Am. J. Physiol., 266*: G31-G39 (1994); Grider et al., *Am. J. Physiol., 264*: G334-G340 (1993); Hellstrom and Ljung, 1996). Both VIP and ATP are also putative inhibitory transmitters of the gut (Smits et al., *Br. J. Pharmacol., 118*: 695-703 (1996); Suthamnatpong et al., *Br. J. Pharmacol., 108*: 348-355 (1993); Yagasaki et al., *J. Pharm. Pharmacol., 35*: 818-820 (1983)).

Interganglionic cholinergic neurons mediate numerous nicotinic excitatory transmissions to initiate and propagate MMCs (Galligan et al., *J. Pharmacol. Exp. Therap., 238*: 1114-1125 (1986)). However, serotonergic transmission is also manifest among enteric neurons (Daniel et al., in Schultz, S.G. and Rauner, B.B., (eds), Handbook of Physiology, pp. 715-757 (Bethesda, Maryland, 1991)). Serotonin (5-HT) also mediates neurogenic stimulation of NANC relaxations and cholinergic contractions of the smooth muscle (Briejer et al., *Naunyn-Schmiedebergs Arch. Pharmacol., 351*: 126-135 (1995); Briejer et al., *J. Pharmacol. Exp. Therap., 274*: 641-648 (1995)). With respect to modulatory influence on motor behavior, 5-HT increases the velocity of MMCs, through neuronal 5-HT_3 receptors (Pineiro-Carrero et al., *Am. J. Physiol., 260*: G232-G239 (1991), Sagrada et al., *Life Sci., 46*: 1207-1216 (1990)).

The focus of this study was to evaluate and compare the pharmacology of the enteric neural pathways controlling interdigestive motor activity in different regions of the rat small intestine *in vivo*. The goal was to determine the extent of cholinergic and 5-HT involvement, as well as the role of ATP, VIP and NO in spontaneous motor activity of the duodenum and ileum.

Surgical Procedure

Male Sprague-Dawley rats (250-350 g) were fasted for 24 hrs with free access to water. For surgery, anesthesia was induced with 2% Halothane in 500 ml•min^{-1} oxygen, and body temperature was maintained constant at 37°C using a thermostatically controlled heated table and a thermal blanket. The right carotid artery was exposed and cannulated to monitor blood pressure via a pressure transducer (P23ID, Gould Statham, OH). The right jugular vein was cannulated for intravenous drug injections. An intra-arterial (i.a.) route of drug administration was often favored, due to the short half-life of many drugs and to evade hepatic first-pass

metabolism. For this, a cannula was inserted from the right femoral artery and fed in a retrograde direction to position the tip at the level of the superior mesenteric artery.

Motility Recording

Animals were prepared for assessment of motility *in vivo* after the method of Krantis *et al.* (1996). Following a median laparotomy, foil strain gauges were sequentially attached using Vet Bond glue onto the anti-mesenteric border of the duodenum, 1-2 cm distal to the gastroduodenal junction; and lateral to the anti-mesenteric border of the ileum, just proximal to the ileocecal junction. In 6 experiments, 2 or 3 foil strain gauges were attached 2 cms apart to the proximal duodenum. From these experiments, we extrapolated the propagation velocity of the 'grouped' activity. All intestinal placed foil strain gauges were oriented parallel to the longitudinal muscle layer since this affords the most sensitive setting for recording circumferential motor activity (Krantis et al., 1996). Wire leads were exteriorized and attached to an IBM PC data acquisition system via a three channel interface box. Rats were then turned over to the prone position, and the concentration of Halothane was lowered to 1% for the remainder of the experiment. Rats were allowed to recuperate from the surgery for one hour, then control motor activity was recorded for another hour before the administration of any drugs. All surgical and experimental protocols were carried out according to the Canadian Council on Animal Care guidelines administered by the Animal Care Committee at the University of Ottawa.

Data Analysis

The protocol for analyzing motility recordings using foil strain gauges is detailed in Krantis et al. (1996). In brief, motor activity was acquired, digitized and stored by an IBM data acquisition system, which calculated, in addition to other variables, the amplitude and frequency

of motor responses. Qualified responses were marked depending on their capacity to satisfy two discriminating sets (for contractions and relaxations) of six numerical parameters. These parameters were monitored over sequential two minute periods and adjusted as deemed necessary to mark motor responses within 95-100% accuracy. Results were then output, and organized into tabular form for statistical analysis.

Statistical Analysis

A one-way ANOVA with a Tukey multiple analysis test was used for comparison between mean values, using Statgraphics Plus 5.0 software. A probability of less than 0.05 ($p<0.05$) was considered significant. All values are expressed as mean ± S.E.M of experiments.

Drug Treatments

All the drugs were dissolved in 0.5 ml of physiological saline (0.9%). The doses (delivered within 1 minute) were: α, β-methylene ATP (300 $\mu g \cdot kg^{-1}$), methyl-S ATP (360 $\mu g \cdot kg^{-1}$), NT-nitro-L-arginine methyl ester (L-NAME, 10 $mg \cdot kg^{-1}$), vasoactive intestinal peptide (VIP, 4-10 $\mu g \cdot kg^{-1}$), BRL 43694 (granisetron, 80 $mg \cdot kg^{-1}$), atropine (4-6 $mg \cdot kg^{-1}$) and hexamethonium (18 $mg \cdot kg^{-1}$ s.c.), all purchased from Sigma, except for α, β-methylene ATP and methyl-S ATP, which were obtained from RBI; Granisetron which was a gift from Dr. R.K. Harding.

Control Recordings

Region specific patterns of spontaneous motor activity were easily characterized in the rat small intestine. In the duodenum (n=8), this consisted of reoccurring cycles of propagating 'grouped' and non-propogating 'intergroup' motor activities, with a cycle length of 5.4±0.4 min. 'Grouped' activity was typified by an intense period (approximately 2-4 min) of contractile and/or relaxant motor activity, that propagated caudally at a rate of 3.4±0.6 $cm \cdot min^{-1}$, in a manner reminiscent of

MMC's. The 'intergroup' activity consisted of randomly occurring, low amplitude, low frequency relaxations and/or contractions.

Spontaneous motor activity of the ileum consisted of either relaxations (50% of all animals tested) or contractions only (30% of all animals tested); in the remaining experiments, contractile and relaxant motor activity occurred together. The predominance of one type of motor response (contraction vs. relaxation) is thought to be indicative of the intrinsic tone of the smooth muscle; where tissue with high tone shows mainly relaxant activity, whilst tissue with low tone more readily shows contractions. Generally, the spontaneous ileal relaxations and contractions occurred at a relatively low frequency, and were of relatively high amplitude. In 10% of the experiments, the ileum displayed periodic bursts of high frequency motor responses comparable to phase III MMC activity.

Effects of P_{2X}- and P_{2Y}-Purinoceptor Agonists on Spontaneous Motor Activity

The substituted derivatives of ATP, α, β-methylene ATP and methyl-S ATP, have differential affinities for P_{2X}- and P_{2Y}-purinoceptors, respectively (Burnstock and Kennedy, *Gen. Pharmacol., 16*: 433-440 (1985)). Tissues develop tachyphylaxis following prolonged exposure to these agents, and thus in this manner it was possible to discriminate between P_{2X} and P_{2Y} receptor-mediated responses (Glasgow et al., 1998). Upon injection, α, β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a.) evoked a phasic relaxation in the duodenum (1.0±0.1 g, n=5); subsequently, it selectively attenuated ($p<0.05$) the frequency and amplitude of 'grouped' relaxations by 73±7% and 48±5%, respectively. The effects of α,β-methylene ATP on spontaneous duodenal contractions were variable and could not be analyzed.

In the ileum, α,β-methylene ATP (300 $\mu g \cdot kg^{-1}$, i.a., n=8) evoked an initial phasic relaxation. Proximate rechallenge with α,β-methylene ATP did not elicit another response,

indicative of the development of tachyphylaxis. During this period, spontaneous ileal relaxations were ($p<0.05$, n=8) attenuated for up to 30 minutes. Spontaneous contractions were not affected by α, β-methylene ATP treatment. Methyl-S ATP (360 $\mu g \cdot kg^{-1}$, i.a., n=4) also attenuated ($p<0.05$) ileal relaxations, however, methyl-S ATP did not evoke an initial phasic relaxation.

Effects of L-NAME on Spontaneous Motor Activity

L-NAME (10 $mg \cdot kg^{-1}$, i.v., n=8), selectively attenuated the frequency and amplitude of spontaneous 'intergroup' relaxations of the duodenum 44±8% and 66±1% respectively. In the ileum, L-NAME potentiated both the contractile (n=6) and relaxant (n=8) motor activity. This effect often persisted for the entire duration of the experiment. The relaxations potentiated by L-NAME were attenuated ($p<0.05$, n=4-6) by either α, β-methylene ATP (by 59±12%) or methyl-S ATP treatment (by 70±3%).

Effects of Muscarinic and Ganglionic Nicotinic Receptor Antagonists

Spontaneous contractions and relaxations of 'grouped' and 'intergroup' motor activity, as well as motor activity of the ileum, were all attenuated ($p<0.05$, n=4) for up to 20 minutes by the nicotinic receptor antagonist hexamethonium (18 $mg \cdot kg^{-1}$, s.c.). L-NAME-enhanced activity was also attenuated ($p< 0.05$, n=6) by hexamethonium. Atropine (4-6 $mg \cdot kg^{-1}$, i.a., n=4), attenuated the spontaneous ileal contractions by 87±3% and 89±7% respectively. Duodenal contractions were similarly affected.

The Role of VIP in the Control of Spontaneous Motor Activity

VIP (4-10 $\mu g \cdot kg^{-1}$, i.a.) evoked a phasic relaxation (n=8) in the duodenum. Subsequently, VIP transiently inhibited ($p<0.05$) duodenal 'intergroup' motor activity, and potentiated ($p<0.05$) the 'grouped' activity. In the ileum, VIP (4-10 $\mu g \cdot kg^{-1}$, i.a.) consistently evoked only a slow contraction which recovered to control level within 6 minutes. Concomitant

with this contraction, spontaneous (n=4) and L-NAME-enhanced (n=6) relaxations were attenuated ($p<0.05$) for up to 8 minutes. The frequency and amplitude of the spontaneous relaxations were reduced to 33±8% and 21±5% of control, respectively. The frequency and amplitude of the L-NAME induced relaxations were reduced to 32±12% and 14±3% of control, respectively.

Effects of the 5-HT_3 Receptor Antagonist Granisetron

Within 5 minutes, granisetron (80 $\mu g \cdot kg^{-1}$, i.v. or i.a.) attenuated ($p<0.05$) spontaneous duodenal 'grouped' relaxations (n=9) and contractions (n=4), but did not affect the 'intergroup' motor activity. The 'grouped' motor activity was reduced for up to 40 minutes; subsequently, the control pattern of interdigestive motility progressively recovered. Granisetron treatment also attenuated ($p<0.05$, n=4) the spontaneous ileal contractions and relaxations. The interdigestive motor pattern of the ileum progressively recovered to control levels within approximately 60 minutes. The amplitude of the L-NAME enhanced ileal motor activity was also attenuated ($p<0.05$, n=6) by 76±8%, in the presence of granisetron.

These findings confirm previous studies (Glasgow et al., 1998; Krantis et al., 1996; Krantis et al., *Am. J. Physiol., 38 (Gastrointest. Liver Physiol., 38)*: G897-G903 (1998)), where relaxations within patterned spontaneous motor activity of the duodenum were found to be dependent upon either NO or ATP. Grouped relaxations were sensitive to α,β-methylene ATP treatment, while "intergroup" relaxations were inhibited in the presence of the NO-synthase inhibitor L-NAME. By contrast, our results show that NO is not the mediator of spontaneous ileal relaxations. Others have shown that in isolated rat ileal preparations, application of ATP evokes relaxations and ATP-desensitization reduces these relaxations (Smits et al., *Br. J.*

Pharmacol., 118: 695-703 (1996)). In addition, α,β-methylene ATP can directly relax smooth muscle (Windschief et al., *Br. J. Pharmacol., 115*: 1509-1517 (1995)), and P_{2X}-purinoceptors are localized on rat intestinal smooth muscle (Katsuragi et al., *J. Pharmacol. Exp. Therap., 259*: 513-518 (1991)). In this study, systemic injection of the P_{2X}-purinoceptor agonist α,β-methylene ATP, induced an initial relaxation of the ileum. A proximate re-administration of α,β-methylene ATP did not induce a response, indicative of the development of tachyphylaxis. Concomitant with the induced tachyphylaxis, spontaneous ileal relaxations were inhibited. Hence, ATP, via P_{2X}-sites, is the transmitter mediating spontaneous NANC relaxations in the rat ileum.

There is *in vitro* evidence that NO and not ATP is a mediator of evoked relaxations in the rat ileum (Kanada et al., *Cell Tiss. Res., 278*: 197-200 (1993); Smits et al., *Br. J. Pharmacol., 118*: 695-703 (1996); Yagasaki et al., 1983). However, in young rats, the primary evoked relaxation in the ileum is partly inhibited by L-NAME and partly by ATP desensitization, while in adult rats, NANC relaxations are only inhibited by ATP desensitization (Smits et al., *Eur. J. Pharmacol., 303*: 79-86 (1996)). Although NO does not mediate spontaneous relaxations of the rat ileum, treatment with L-NAME potentiates spontaneous ileal relaxations and contractions. This strongly suggests that interdigestive motor activities of the rat ileum are under tonic nitrergic inhibition. Mathias et al. (*Am. J. Physiol., 249*: G416-G421 (1985)) had previously demonstrated that spontaneous contractions in the rat ileum are under tonic inhibitory control, and the notion of a general tonic inhibition in the gut was first proposed by Wood (*Am. J. Physiol., 222*: 118-125 (1972)) and confirmed by others who showed that nerve blocking agents evoked contractile activity in the gut *in vitro*, and intestinal hypermotility *in vivo* (Delbro, *News Physiol. Soc., 11*: 67-71 (1996)). There is accumulating evidence for NO to be the transmitter of

this tonic inhibition (Caligano et al., *Eur. J. Pharmacol., 229*: 273-276 (1992); Daniel et al., 1994; Gustafsson and Delbro, *J. Aut. Nerv. Sys., 44*: 179-187 (1993)).

NO modulated propagatory motor activity of the rat duodenum (Glasgow et al., 1998) confirming results in isolated segments of rat duodenum (Postorino et al., 1995). Other *in vivo* studies show NO-synthase inhibitors to shorten the MMC cycle length, induce premature MMCs, potentiate phase III activity, reduce the quiescent phases of MMCs, and increase motility of the rat intestine (Calignano et al., 1992; Hellstrom and Ljung, 1996; Sarna et al., 1993). Thus NO functions within the enteric neural circuits, particularly in the control of stereotypic patterns of interdigestive motility and intestinal transit.

ATP exhibits multiple enteric neural functions, since in addition to mediating P_{2X}-purinoceptor dependent relaxations in the duodenum and ileum, ATP via P_{2Y}-purinoceptors can stimulate NO-mediated non-propagating 'intergroup' relaxations in the duodenum (Glasgow et al., 1998). In the present example, the P_{2Y}-purinoceptor agonist, methyl-S ATP, inhibited spontaneous ileal relaxations. However, in contrast to α,β-methylene ATP, methyl-S ATP did not evoke an ileal relaxation upon injection. This suggests that P_{2Y}-purinoceptors are not present on the smooth muscle, or else, are not active within the inhibitory motor innervation(s) of the ileum. The data support the view that in the rat ileum, P_{2Y}-purinoceptors are involved in the activation of pathways mediating tonic inhibition of the purinergic NANC motor neurons targeting P_{2X} purinoceptors. P_{2Y}-purinoceptors may be present on nitrergic interneurones subserving tonic inhibition, or on other interneurones within this prejunctional input. The nitrergic and purinergic interneurones may also represent the same population, since ATP and NO synthase are colocalized in myenteric neurons in the rat ileum (Belai and Burnstock, *Cell Tiss. Res., 278*: 197-200 (1994)).

VIP is a NANC inhibitory transmitter in numerous gut regions (Bojo et al., *Eur. J. Pharmacol., 236*: 443-448 (1993); Mule et al., *J. Auton. Pharmacol., 12*: 81-88 (1992); Suthamnatpong et al., 1993)). In these experiments, VIP evoked a transient relaxation in the rat duodenum. The subsequent development of tachyphylaxis to VIP inhibited the contractile and relaxant "intergroup" activity and enhanced the "grouped" motor activity. The data indicate that the initial VIP-evoked relaxations are dependent on NO and sensitive to VIP desensitization (Krantis et al., 1998). Thus VIPergic interneurones must be targeting direct motor innervations (the nitrergic and cholinergic motor neurons) of the "intergroup" activity, as well as the nitrergic prejunctional modulatory inputs of the "grouped" activity. Indeed, VIP is known to stimulate both cholinergic and NANC motor activity via neurogenic pathways in the guinea-pig (Katsoulis et al., *Regulatory Peptides, 38*: 155-164 (1992); Kusunoki et al., *Am. J. Physiol., 251*: G51-G55 (1986)). Moreover, VIP binding sites were localized in high densities in the myenteric nerve layer (King et al., *Peptides, 10*: 313-317 (1989); Sayadi et al., *Peptides, 9*: 23-30 (1988)) but not on the smooth muscle.

In these experiments, treatment with VIP (Yagasaki et al., 1983) inhibited the spontaneous relaxations of the ileum. Since VIP did not evoke a relaxation upon injection, it is unlikely that VIPergic neurons mediate direct inhibitory input to the ileal smooth (Smits et al., *Br. J. Pharmacol., 118*: 695-703 (1996)). *In vivo* experiments in the canine ileum, suggest that VIP plays a major role in the tonic inhibition of circular muscle motor activity via an inhibitory neural action (Fox-Threlkeld et al., *Peptides, 12*: 1039-1045 (1991)). The data support the view that VIP targets the NO-dependent prejunctional modulation of the purinergic inhibitory motor innervations in both the duodenum and ileum. Interestingly, these purinergic motor pathways specifically generate the propagatory motor activity of the small intestine. Furthermore, VIP specifically inhibits phase III activity of MMCs, and VIP antagonists initiate phase III activity (Hellstrom and Ljung, 1996).

Although VIP treatment evoked a relaxation only in the duodenum, VIP also evoked a contraction in the rat ileum. Contractions to VIP have been described before in the rat ileum (Smits et al., *Br. J. Pharmacol., 118*: 695-703 (1996)) where it was determined these contractions were neurally mediated and involved a cholinergic and a substance P component. It is intriguing that the duration of the VIP-evoked contraction in this study corresponds to the period of VIP-mediated inhibition of spontaneous relaxations. The conclusion is that VIP simultaneously stimulates excitatory motor inputs and inhibitory nitrergic prejunctional inputs of the purinergic motor neurons in the rat ileum.

VIP often coexists with NO synthase in neural elements of the myenteric plexus, particularly in the profuse terminal axonal networks that extend to other myenteric ganglia and smooth muscle (Ekblad et al., *Neurosci., 63*: 233-248 (1994)). VIP and NO are thought to work together as inhibitory mediators but by different cellular mechanisms, or else, the release of one affects the other (Daneil et al., 1994; Grider, 1993; Hellstrom and Ljung, 1996). *In vivo* experiments show that VIP stimulates NO mediated duodenal relaxations via GABAergic interneurones (Krantis et al., 1998). Thus, VIP mediated motor and neuromodulatory pathways represent an important integrative component of enteric neural circuits in this gut region.

All components of the interdigestive motor complex are dependent upon vago-sympathetic integrity (Chung et al., *Am. J. Physio., 267*: G800-G809 (1994); Galligan et al., *J. Pharmacol. Exp. Therap., 238*: 1114-1125 (1986)). The cholinergic interneurones controlling gastrointestinal motility, act via nicotinic synapses, whereas ACh (acetyl choline) actions on smooth muscle are via muscarinic receptors (Galligan et al., 1986). Atropine inhibited all spontaneous contractions of the small intestine. With respect to the control of nitrergic interneurones, it has been shown that NO is produced in enteric ganglia in response to nicotinic

receptor agonists (Makhlouf and Grider, *NIPS, 8*: 195-199 (1993)). The results from this study indicate that all of the spontaneous interdigestive motor activity of the duodenum and ileum is tonically driven, and is sensitive to nicotinic receptor blockade. This was expected since nicotinic ganglionic transmission mediates all MMC activity in the canine, guinea-pig and rat (Galligan et al., 1986).

In vitro studies show neuronally derived 5-HT stimulates purinergic NANC relaxations (Briejer et al., *Naunyn-Schmiedebergs Arch. Pharamacol., 351*: 126-135 (1995); Briejer et al., *J. Pharmacol. Exp. Therap., 274*: 641-648 (1995)), and cholinergic contractions (Briejer et al., *Eur. J. Pharmacol., 308*: 173-180 (1996)). The *in vivo* results in this study show that 5-HT_3 receptors are involved within the motor pathways mediating the spontaneous cholinergic contractions and purinergic relaxations in the duodenum and ileum. These findings corroborate other *in vivo* studies (Pineiro-Carrero et al., 1991; Sagrada et al., *Life Sci., 46*: 1207-1216 (1990)).

An arrangement of cholinergic, nitrergic, GABAergic, purinergic and VIPergic neural elements within the proposed tonic and modulatory pathways controlling spontaneous motor activity in the rat duodenum and ileum is presented schematically in the simplified wiring diagram of Fig. 10. Stereotypic motility patterns are elicited when driver circuits activate excitatory and inhibitory motor pathways, as determined by 'enteric neural programs'. However, a continuous drive from inhibitory interneurones maintains myogenic activity quiescent. This coordinated inhibition and disinhibition is mediated by the inhibitory nitrergic inputs, and that it is precisely the control of these prejunctional neuronal pathways, along with the tonically active motor pathways, which generates cyclical (interdigestive) motility patterns upon an existing baseline of motor activity.

All publications cited in the text are incorporated herein by reference.

What is claimed is:

1. A method of treating obesity in an animal comprising administering to said animal a non-toxic, gut motility-regulating amount of a trichothecene.

2. The method of treating obesity according to claim 1 wherein the trichothecene is selected from the group consisting of deoxynivalenol, nivalenol, trichothecolon, trichothecin, 3-acetydeoxynivalenol, 7-acetyldeoxynivalenol, 3,15-diacetyldeoxynivalenol, 4-acetylnivalenol(fusarenon-X), and 4,15-diacetylnivalenol.

3. The method of treating obesity according to claim 2 wherein the trichothecene is deoxynivalenol.

4. The method of treating obesity according to claim 1 wherein the trichothecene is administered orally, parenterally, intravenously, intramuscularly, or intra-arterially.

5. The method of treating obesity according to claim 4 wherein the trichothecene is administered orally.

6. The method of treating obesity according to claim 1 wherein the animal is selected from the group consisting of primates, swine, cattle, sheep, poultry and other birds, horses, cats, dogs, and rodents.

7. The method of treating obesity according to claim 1 wherein the animal is a human.

8. A method of stimulating the fed pattern of gut motility in an animal comprising administering to said animal a non-toxic, gut motility-regulating amount of a trichothecene, a trichothecene analog, or a non-desensitizing agonist of the P_{2X1} receptor.

9. The method of stimulating the fed pattern of gut motility in an animal according to claim 8, wherein the trichothecene is selected from the group consisting of deoxynivalenol, nivalenol,

trichothecolon, trichothecin, 3-acetydeoxynivalenol, 7-acetyldeoxynivalenol, 3,15-diacetyldeoxynivalenol, 4-acetylnivalenol(fusarenon-X), and 4, 15-diacetylnivalenol.

10. The method of stimulating the fed pattern of gut motility in an animal according to claim 8, wherein the trichothecene is deoxynivalenol.

11. The method of stimulating the fed pattern of gut motility in an animal according to claim claim 8, wherein the trichothecene is administered orally, parenterally, intravenously, intramuscularly, or intra-arterially.

12. The method of stimulating the fed pattern of gut motility in an animal according to claim 8, wherein the trichothecene is administered orally.

13. The method of stimulating the fed pattern of gut motility in an animal according to claim 8, wherein the animal is selected from the group consisting of primates, swine, cattle, sheep, poultry and other birds, horses, cats, dogs, and rodents.

14. The method of stimulating the fed pattern of gut motility in an animal according to claim according to claim 8, wherein the animal is a human.

15. The method of stimulating the fed pattern of gut motility in a vertebrate animal according to claim 8, wherein the non-desensitizing agonist of the P_{2X1} receptor is an analog of adenosine triphosphate.

16. A method of increasing weight in an animal comprising administering to said animal an analog of adenosine triphosphate.

17. The method of increasing weight in an animal according to claim 16, wherein the analog of adenosine triphosphate is a desensitizing agonist or an antagonist of the P_{2X1} purinoceptor.

18. The method of increasing weight in an animal according to claim 17, wherein the desensitizing agonist of the P_{2X1} receptor is α,β-methylene ATP.

19. A method of stimulating the fasting pattern of gut motility in an animal comprising administering an analog of adenosine triphosphate.

20. The method of stimulating the fasting pattern of gut motility in an animal according to claim 19, wherein the analog of adenosine triphosphate is a desensitizing agonist or an antagonist of the P_{2X1} receptor.

21. The method of stimulating the fasting pattern of gut motility in an animal according to claim 20, wherein the desensitizing agonist of the P_{2X1} receptor is α,β-methylene ATP.

22. A method of identifying a compound for treating obesity comprising the steps of:

detecting the ability of the compound to inhibit protein synthesis; and

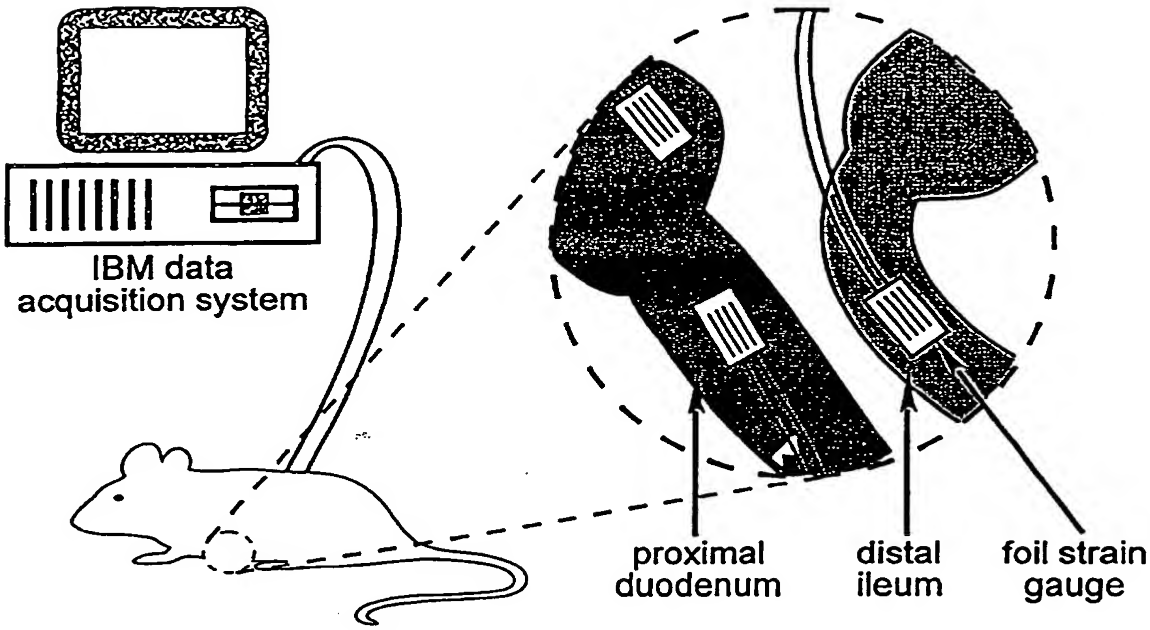
determining whether the compound capable of inhibiting protein synthesis is also capable of stimulating the fed pattern of gut motility.

23. The method of identifying a compound for treating obesity according to claim 22, wherein the compound is tested for the ability to stimulate the fed pattern of gut motility using an *in vitro* gut organ bath assay, an *ex vivo* gut organ assay, or an *in vivo* assay for gut organ motility.

Abstract of the Invention

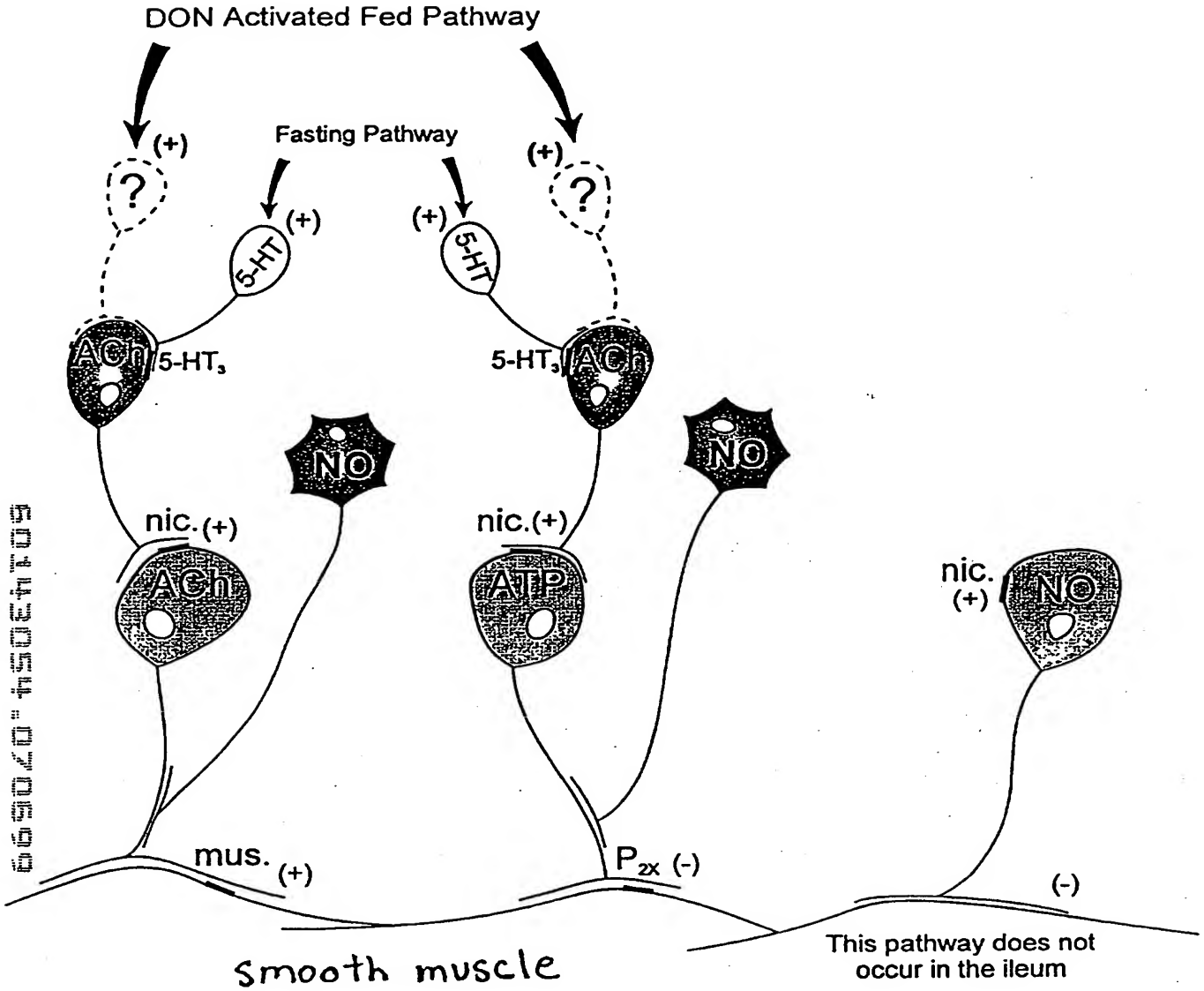
Methods and compositions for regulating gut motility and food intake are described. Such methods may be used to treat obesity or to stimulate weight gain in an animal.

Fig. 1



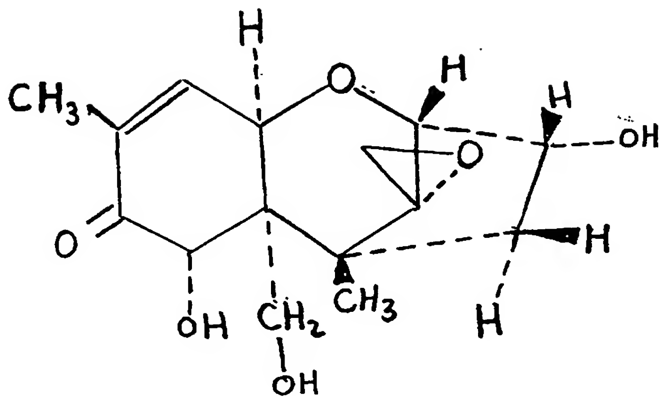
A schematic of the *in vivo* setup employed for recording gut motility in rats. Foil strain gauges are glued onto the serosal surface of the gastric antrum, duodenum and ileum, along the longitudinal muscle layer. The wire leads are attached to an IBM data acquisition system.

Fig. 2



Pathways controlling stereotypic gut motility patterns in the duodenum and ileum. Propagatory motor activity is mediated by cholinergic (excitatory) and purinergic (inhibitory) motor neurons. Nitrergic interneurones tonically modulate both the excitatory and inhibitory pathways. Both motor pathways contain cholinergic and serotonergic interneurones. However serotonergic interneurones only mediate the fasting motor pattern. DON (from a site outside of the gut) activates the fed pattern via a pathway independent of 5-HT_3 receptors.

Fig. 3



4-Deoxynivalenol

Fig. 4

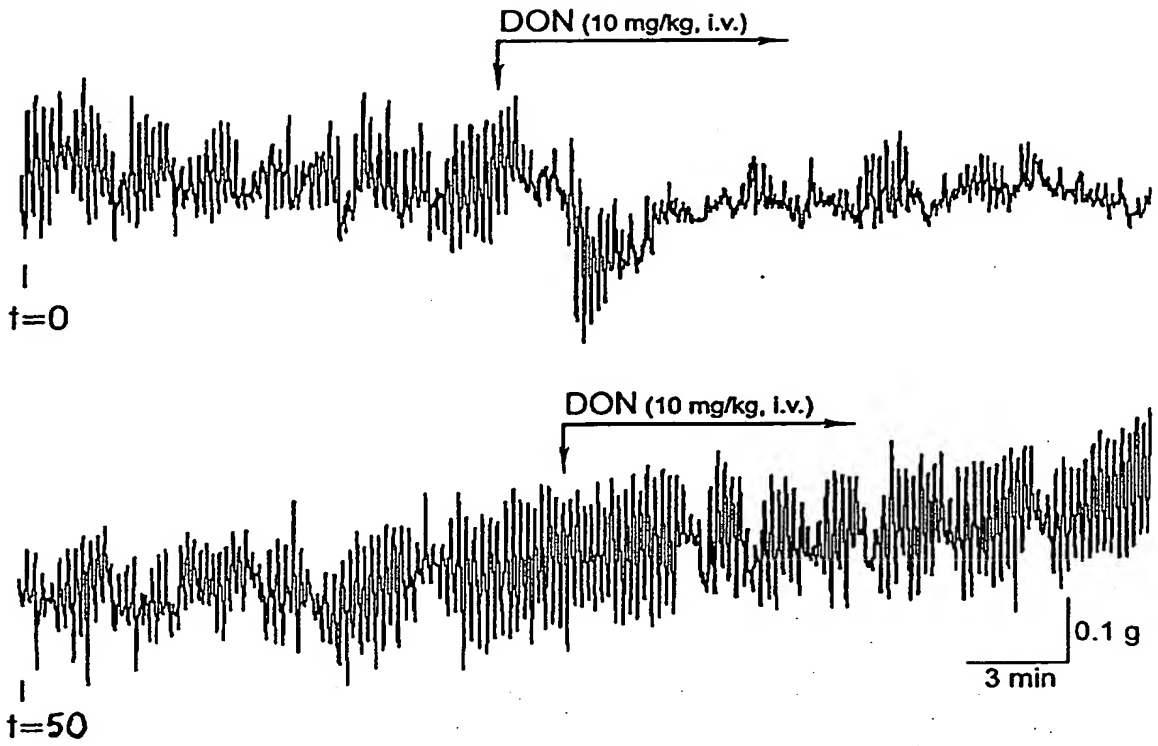


Fig. 5

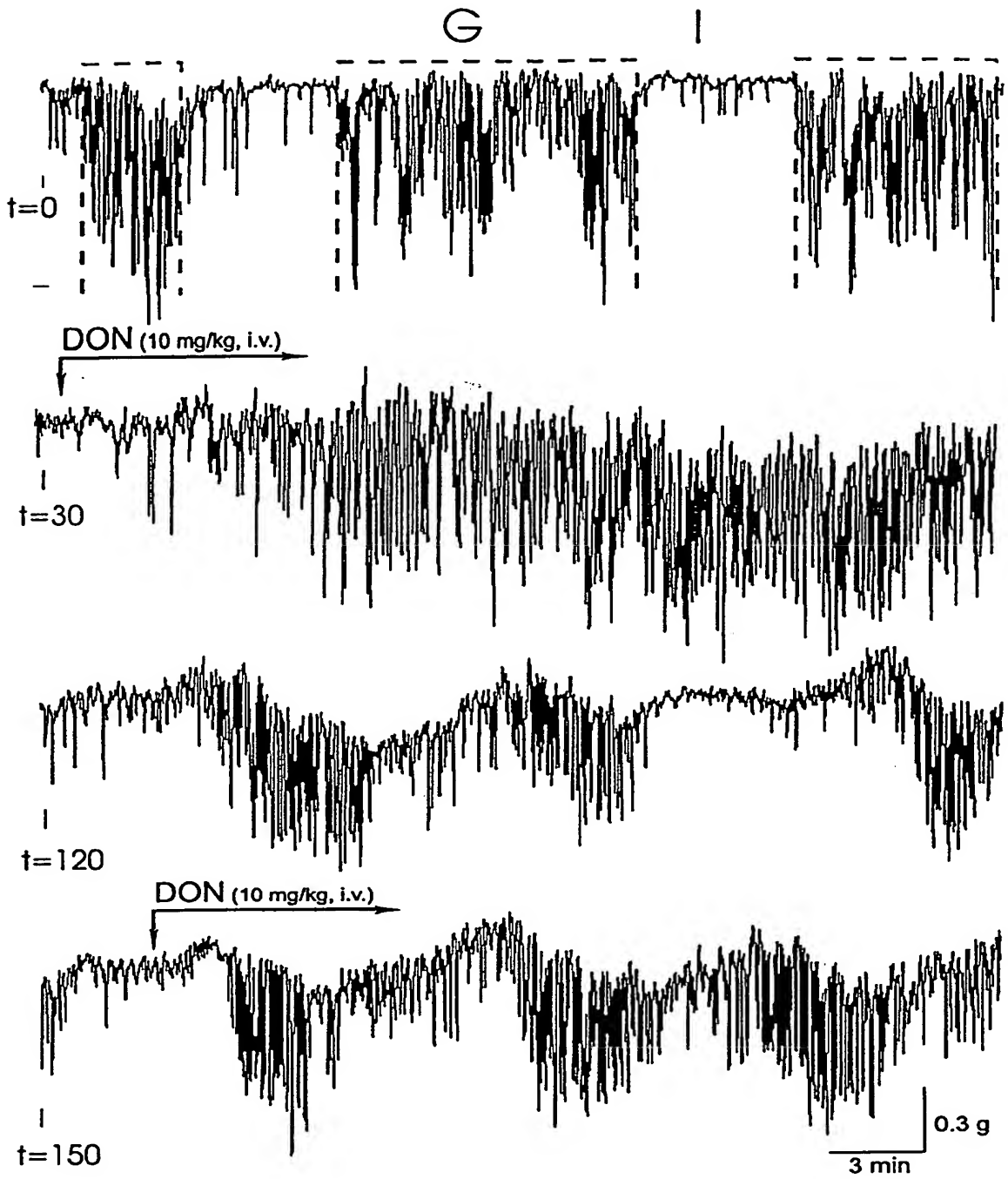


Fig. 6A

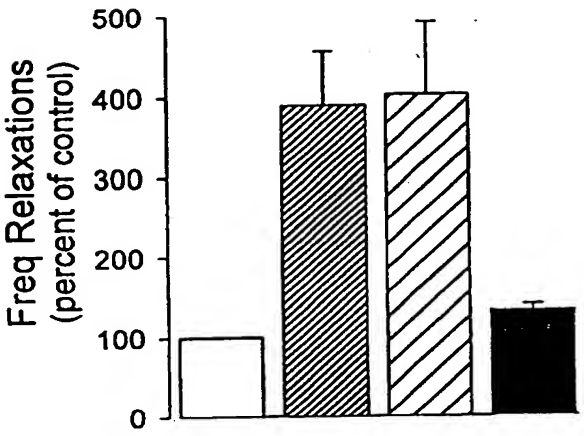

Freq Relaxations
(percent of control)
500
400
300
200
100
0

Fig. 6B

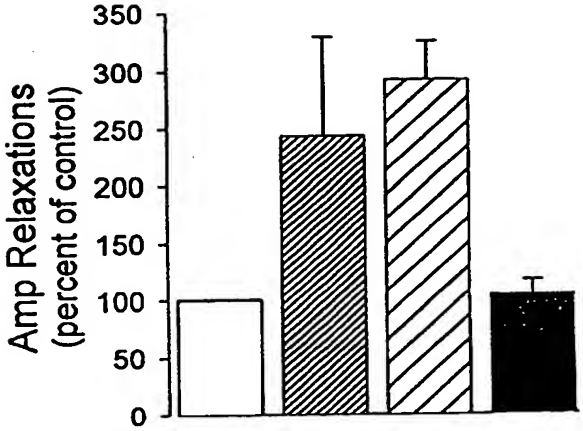

Amp Relaxations
(percent of control)
350
300
250
200
150
100
50
0

Fig. 6C

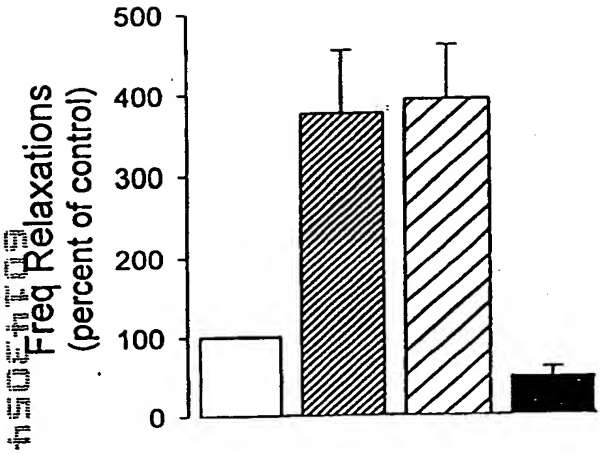

Freq Relaxations
(percent of control)
500
400
300
200
100
0

Fig. 6D

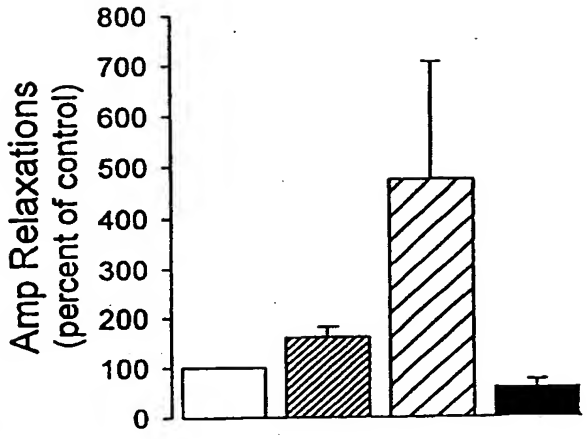

Amp Relaxations
(percent of control)
800
700
600
500
400
300
200
100
0

Fig. 7A

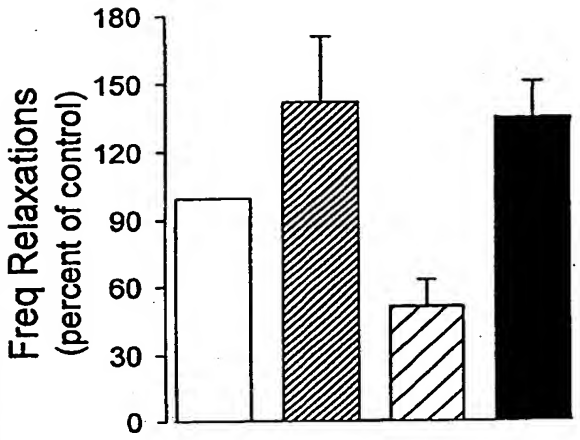

Freq Relaxations
(percent of control)
180
150
120
90
60
30
0

Fig. 7B

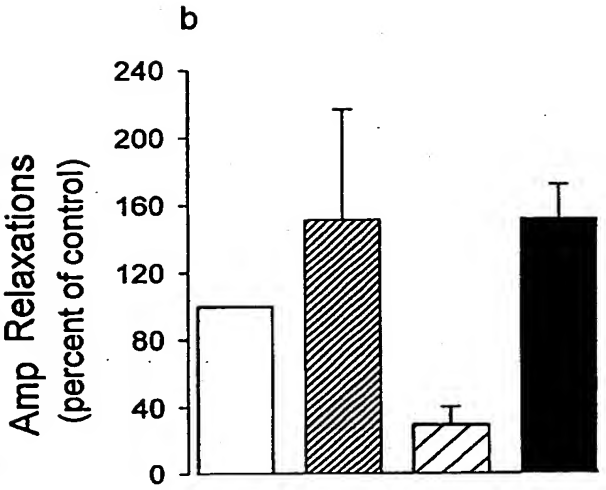

b
Amp Relaxations
(percent of control)
240
200
160
120
80
40
0

Fig. 7C

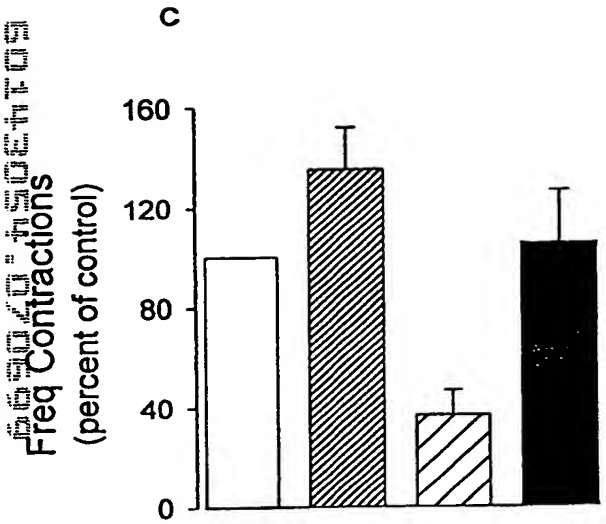

c
Freq Contractions
(percent of control)
160
120
80
40
0

Fig. 7D

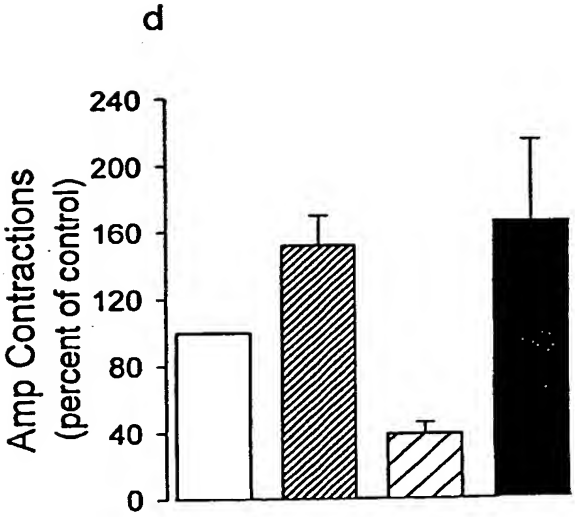

d
Amp Contractions
(percent of control)
240
200
160
120
80
40
0

Fig. 8

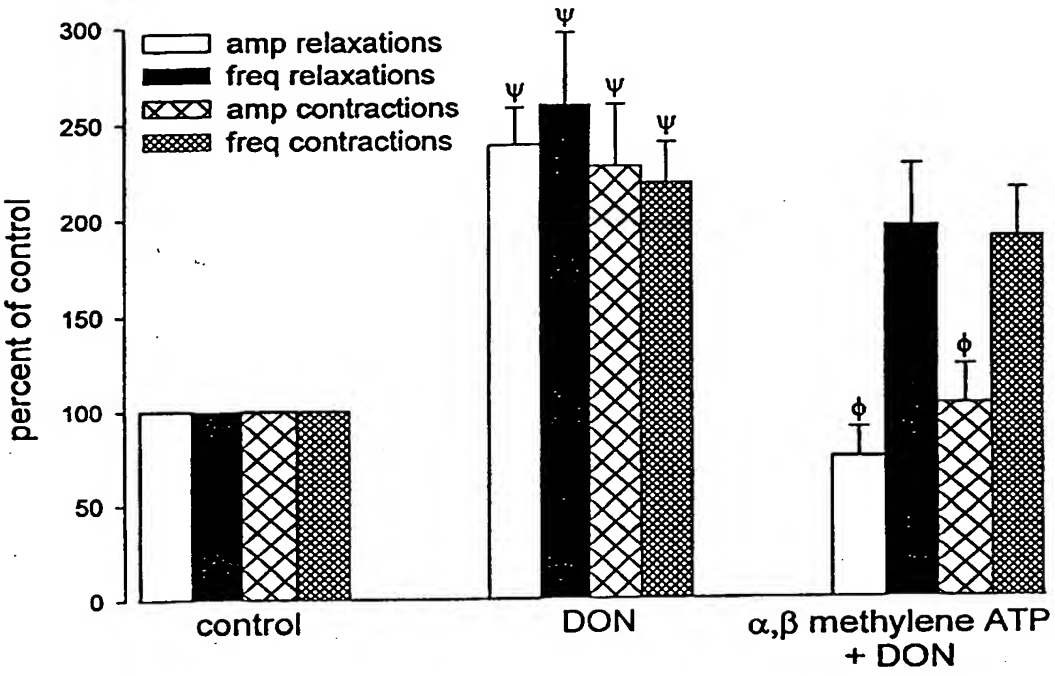

amp relaxations
freq relaxations
amp contractions
freq contractions
percent of control
300
250
200
150
100
50
0
control
DON
α,β methylene ATP
+ DON

Fig. 9

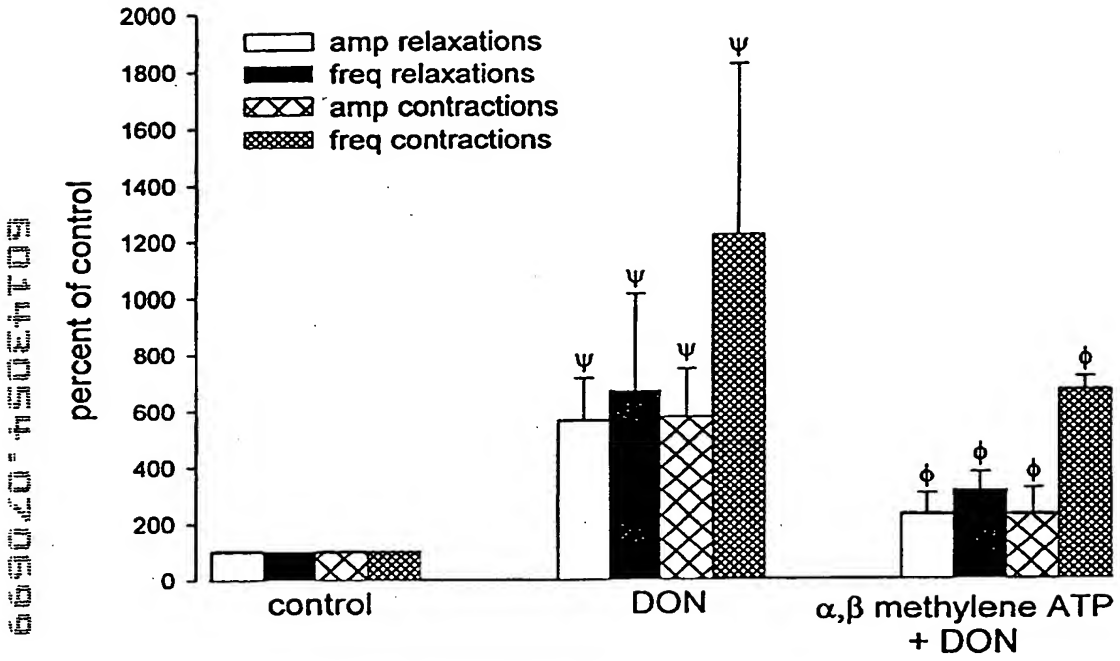
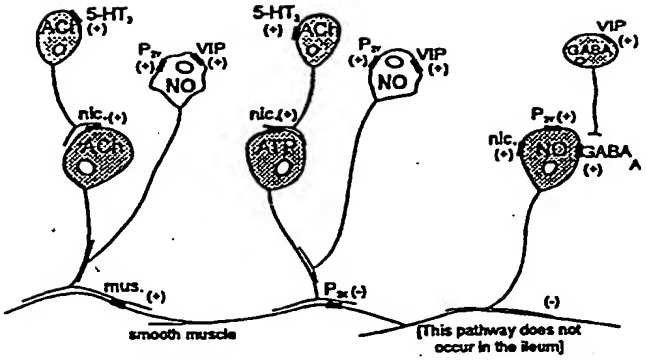

amp relaxations
freq relaxations
amp contractions
freq contractions
percent of control
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Ψ
Ψ
Ψ
Ψ
ϕ
ϕ
ϕ
ϕ
control
DON
α,β methylene ATP
+ DON

Fig. 10


5-HT3
(+)
ACh
P2Y
(+)
VIP
(+)
NO
nic.(+)
ACh
mus.(+)
smooth muscle
5-HT3
(+)
ACh
ATP
P2Y
(+)
VIP
(+)
NO
P2Y(-)
VIP
(+)
GABA
P2Y(+)
nic.
(+)
NO
GABAA
(+)
(-)
[This pathway does not occur in the ileum]